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ABSTRACTS OF PAPERS PRESENTED AT THE 1960 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

STILLWATER, OKLAHOMA, AUGUST 29 TO AUGUST 31, 1960

AHLOOWALIA, B. S., and E. D. GARBER, University of Chicago, Chicago, Ill.: *The genus Collinsia. Reciprocal translocations in the interspecific hybrids between C. bruceae, C. solitaria and C. sparsiflora arvense.*—Hybrids between *C. solitaria* and *C. bruceae* or *C. sparsiflora arvense* had two rings of four chromosomes and three pairs at metaphase I; hybrids between *C. bruceae* and *C. sparsiflora arvense* had seven bivalents. The rings displayed an alternate distribution at this stage and were considered to be interchange complexes. All hybrids were fertile and those with rings yielded progeny with bivalents, one or two rings in a ratio of 1:2:1. It was not possible to detect differences in the fertility of plants from each category. Species, hybrids, and their progeny had essentially the same mean number of chiasmata at metaphase I. (Aided by the National Science Foundation and Abbott Memorial Fund, University of Chicago.)

ALEXANDER, MARY L., Biology Department, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas: *Dose rate effects and genetic recovery with cobalt-60 γ-rays in atmospheres of oxygen and nitrogen.*—Mature males of *Drosophila virilis* were treated with cobalt-60 in atmospheres of oxygen or nitrogen using dose rates of 2000r/min, fractionated doses and slow rates of 50 or 100r/min. Genetic recovery was tested with comparative values for dominant lethals and translocations at different dose rates. Treatments in oxygen and nitrogen, different total doses and combination treatments in oxygen and nitrogen were used.—In mature sperm there were no indications of genetic recovery, measured as dominant lethals or translocations, with dose rate changes. Results for dominant lethals in nitrogen were 17.4% for 2000r at a fast rate, 16.7% for doses fractionated for 30 minutes and 16.9% for slow dose rates. With 4000r the percentages for dominant lethals were 53.6, 51.4 and 57.4% for fast, fractionated and slow rates. Translocation values in the latter tests were 8.8, 7.9 and 10.9% for fast, fractionated and slow rates. Atmospheres of pure oxygen, as with anoxia, did not affect genetic recovery with values of 26.5, 26.3 and 27.8% translocations being obtained for the three tests.—Tests with combined treatments included (1) oxygen-oxygen (2) oxygen-nitrogen (3) nitrogen-oxygen and (4) nitrogen-nitrogen. Results for 1500r + 1500r γ-rays were (1) 67.8% (2) 50.8% (3) 53.5% and (4) 51.1% dominant lethals. These tests do not indicate preferential breakage in oxygen with failure to heal (restitution) more than breakage in nitrogen since the values for (2) and (3) are too low. The amount of genetic damage, which would indicate genetic recovery, could not be affected by varying the dose rates in anoxic or oxygenated atmospheres using mature sperm of Drosophila. (Work supported by contract AT-(40-1)-1828 with the United States Atomic Energy Commission.)

ALLEN, COURTNEY P., Hy-Line Poultry Farms, Johnston, Iowa: *A specific isoimmune chicken antiserum which identifies A locus alleles and B locus heterozygotes within leghorn lines.*—Generally a specific isoimmune chicken antiserum will not agglutinate the red blood cells of chickens produced from a mating in which both parents are negative to the antiserum. An antiserum has been produced within the leghorn that does not agglutinate the parents but can agglutinate the progeny. This antiserum was produced in an inbred line and used as a specific-*A* locus reagent. In a second inbred line it agglutinates only the *B* locus heterozygote B^1B^2.—When B^1B^1 birds are mated with B^2B^2 birds within the second line, the progeny are agglutinated by the antiserum. Absorbing the antiserum with red blood cells from either parent did not remove the antibodies reactive with the heterozygous progeny nor did this absorption weaken the ac-

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tivity of this antiserum for appropriate *A* blood cells. Absorption of the antiserum with *A* antigens of the first line did remove the *A* activity but did not reduce the heterozygous *B* locus activity. The only absorptions found to be capable of removing this antibody utilized the cells of the B^1B^2 heterozygotes and when this was done the *A* antibodies were not removed.—There is no evidence to show if the *B* heterozygote antibodies were normally present or induced by the production of *A* antibodies. Mechanisms for the production of such unusual antibodies cannot be shown.

ALLEN, SALLY LYMAN, University of Michigan, Ann Arbor, Mich.: *Inherited variations in the esterases of Tetrahymena.*—Strain differences in the esterases of Variety 1 of *T. pyriformis* are observed following application of histochemistry to proteins separated by starch gel electrophoresis. Two groups of electrophoretically distinct components (B, C) are found in different inbred strains. Each group is strictly inherited within the clone during asexual or sexual reproduction. Crosses between B and C clones lead to the occasional production of hybrids with both groups of enzymes. Such clones during vegetative reproduction show intraclonal variability, but the rate of change with fissions is small and is not affected by starvation or cold treatment. Subclones "pure" for B or for C can be selected. When B + C clones are intercrossed, three types of offspring appear in the F_2: B + C, B and C, the latter two types breeding true. Initially exconjugants from the same pair are phenotypically alike. F_1 clones of different final phenotype, crossed in various combinations, give rise to all three types of offspring, but at present it is not clear if the ratios are similar in different crosses. Backcrosses to B yield only B and B + C progeny (some of these C in phenotype). These data suggest that the two groups of esterases are under the control of a single locus, but in hybrids nuclear differentiation results in the expression of only one of the two groups. The contribution of the "cytoplasm" in this system is not clear, and certain other crosses are difficult to explain. Tests to date suggest that the esterase locus *may* be linked with the mating type locus. (Supported by a grant from the U.S. Public Health Service.)

ALTENBURG, EDGAR, and LUOLIN S. BROWNING, Rice University, Houston, Texas: *Photoreactivation of mutations induced in Drosophila by different doses of ultraviolet light.*—The polar cap cells (early germ track) in the developing eggs of Drosophila were treated with three different doses of ultraviolet. One series of eggs received no further treatment; a second series was post-treated with photoreactivating light. All three doses were on the part of the dose-frequency curve which shows no further rise in mutation rate with increasing dose of ultraviolet. The mutation rates were based on the percent of lethals detected in the second chromosome by Muller's sifter technique. Controls (shielded from the ultraviolet) gave the usual low mutation rate ($0.4 \pm 0.2\%$) as did also those which were shielded but received photoreactivating light ($0.4 \pm 0.3\%$). In the ultraviolet treated series it was found that although the photoreactivating light may have reduced the mutation rate somewhat in polar caps receiving the lowest ultraviolet dose (from $5.0 \pm 1.6\%$ to $3.0 \pm 1.0\%$) and in those receiving the medium dose (from $4.6 \pm 1.5\%$ to $2.4 \pm 0.6\%$), there is even less indication of a reduction in those receiving the highest dose (from $4.8 \pm 1.5\%$ to $4.1 \pm 0.7\%$). In explanation, it has been suggested that photoreactivating light causes the mutagenically effective dose of ultraviolet to be shifted to the left on the dose-frequency curve. Since this curve is rising at the lower doses but falling somewhat at the higher, a shift to the left would cause a lowering in the mutation rate at the lower doses but not at the higher doses. (This work has been supported by a grant from the National Institutes of Health, U. S. Public Health Service.)

ALTENBURG, EDGAR, and L. S. BROWNING, Rice University, Houston, Texas: *The relative proportion of whole-body mutations versus fractionals in Drosophila.*—Mutations induced by X-rays (3,000r) in Drosophila sperm were studied for relative frequency of whole-body *versus* fractionals in F_1 females heterozygous for 13 visible loci in Muller's Maxy stock. Among a total of 38,000 F_1 females, 14 were proved whole-body and three were fractionals. Also 17 additional presumed whole-body mutants occurred but were not genetically proved. No visible mutations associated

with lethals are included. By contrast, among the controls (representing spontaneous mutations), 24 were fractionals and only six were whole-body among 150,000 F_1 females (a ratio in substantial agreement with SCHALET's). Probably many of the spontaneous fractionals (possibly all) arose in early cleavage stages of the F_1 females and were not actually fractionals in the sense of involving only one strand of a DNA molecule. In a chemically treated series (adult males treated), the fractionals greatly outnumbered the whole-body (12 fractionals and three whole-body among 24,000 F_1 females from phenylalanine mustard treatment, 17 fractionals and three whole-body among 17,000 F_1 females from di-methyl myleran, and eight fractionals and four whole-body among 31,000 F_1 females from 2,5 bis-ethyleneimino hydroquinone). But here the fractionals probably represent delayed effects for the most part. Even in the case of the X-ray induced, apparent fractionals might often represent delayed mutations rather than actual fractionals. The evidence then indicates that true fractionals (involving only one DNA strand) are at best questionable. The preponderance of whole-body mutations induced by X-ray treatment poses a problem in connection with the Watson-Crick model, since presumably only one strand of the double helix would as a rule be changed at a given level by X-rays; or, if two, not both in a complementary manner. (Work supported by a grant received from the National Institutes of Health, United States Public Health Service.)

BALBINDER, E., (Introduction by M. DEMEREC), Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, N.Y.: *Mapping, complementation, and mutation studies with two tryptophan loci of Salmonella typhimurium.*—Eighteen *tryD* and six *tryC* alleles, of spontaneous or mutagen-induced origin, have been analyzed in *S. typhimurium.* These two loci are part of a four-locus sequence (*tryA–B–C–D*), and control the enzyme tryptophan synthetase. —Transduction experiments showed that mutations at 15 sites of the *tryD* locus and six sites of the *tryC* locus are responsible for the 24 mutants. The map order of the mutant sites were determined by three-point recombination tests involving two *tryD* alleles and a *tryB* marker, or two *tryC* alleles and a *tryA* marker. Studies of the recombination frequencies indicated that they are influenced by factors other than distance between the markers involved.—Complementation tests by abortive transduction showed that the 18 *tryD* mutants constitute one complementation group and the six *tryC* mutants another: all *tryC* mutants complement all *tryD* mutants, and *vice versa.* These results agree well with YANOFSKY's finding of two components for tryptophan synthetase in *Escherichia coli,* each component being determined by one of two adjacent loci.—In studies of reversions after treatment with 5-bromouracil, 2-aminopurine, diethyl sulfate, beta-propiolactone, and ultraviolet radiation, each of the 24 mutants showed a distinctive pattern of response. No relation was found between the map position of a mutant and its response to the mutagens. In two cases, mutants belonging to the same site exhibited identical reversion patterns. A third case was found in which two mutants of the same site had different reversion patterns.—Suppressor mutations were obtained in some of the mutants. These are allele specific as far as has been determined. In some cases, strain differences in suppression of the same mutant gene were demonstrated. For example, with the marker *tryD-7* in strain LT7, only one type of suppressor was found (as determined by colony size on minimal agar), but when *tryD-7* was introduced into strain LT2 by transduction, two distinct suppressor types were observed.

BARNETT, W. EDGAR, and A. GIB DEBUSK, Florida State University, Tallahassee, Florida: *Nitrous acid induced reverse mutation in Neurospora crassa.*—The specific mutagenic action of nitrous acid has been described by SCHUSTER and SCHRAMM (1958). More recently, the kinetics of nitrous acid mutation induction have been used to determine the number of copies of the genetic material present in phage and microorganisms. The authors have investigated the induction of reverse mutation in auxotrophic strains of *Neurospora crassa* using the back mutation assay technique of GILES (1951). In particular, an adenine requiring strain (*ad-3B*) of ultraviolet origin has been used. The kinetics of the reverse mutation process indicate the physical dimensions of the mutated region. Thus far, testcrosses with revertant types have not demonstrated the presence of suppressor mutations. These preliminary results indicate that the nitrous acid reverse mutation

process may furnish a useful tool for the determination of the dimensions in nucleotides of many point mutations.

BARRY, EDWARD G., Department of Biological Sciences, Stanford University, Stanford, California: *Genetic analysis of an insertional translocation in Neurospora crassa.*—The arginine requiring strain S1229Ab appears, on genetic and cytological evidence, to contain rearrangements in four chromosomes, corresponding to linkage groups I, II, IV and VII. Among progeny of crosses of S1229Ab by wild type, random separation of one component of the rearrangement complex occurs. This is identified as a small insertional duplication. Isolates carrying the duplication have very low fertility and a decreased growth rate.—Genetic analysis shows that the duplication covers five loci, including *arg-2*, *col-4*, *me-1*, and *pt*(NS1). These lie in a region of group IV between *pdx-1* and *pyr-3*. This region of about five map units long has been inserted into linkage group VII between the markers *me-7* and *thi-3*.—Isolates with the duplication have a standard group IV, and there is probably no additional association of the alteration with another linkage group. In contrast the S1229Ab isolates not only have this fragment of IV inserted in VII but also the remainder of this arm of group IV shows an association with markers of group II.—Cytological observations indicate that the small duplication has profound effects on the events at meiosis in crosses of duplication by wild type. Irregular pairing occurs and univalents are frequently observed at metaphase I. (Supported by Public Health Service research training grant 2G-158.)

BARTLETT, ALAN C., DORIS SHIDELER and A. E. BELL (Introduced by W. H. KYLE), Purdue University, Lafayette, Indiana: *Two loci controlling body color in Tribolium castaneum.*—The genetic relationship between two mutants affecting the red-brown adult body color of the flour beetle, *Tribolium castaneum*, was investigated in F_1, F_2, and backcross progenies. One mutant, black (*b*), is due to a semidominant autosomal factor which produces a black phenotype when homozygous (*bb*) and a distinguishable bronze color when heterozygous (*Bb*), as reported by STANLEY and SLATIS (1955, Ecology 36:473–485). The second mutant, sooty (*s*), is characterized by a dark brown body color and is inherited as an autosomal recessive factor. The F_1 progeny were similar in color to the bronze heterozygous individuals. The phenotypic frequencies observed in the progeny of the F_1 backcrossed to sooty were 127 red, 131 bronze and 258 sooty. F_1 progeny backcrossed to black gave frequencies of 115 bronze to 97 black. F_2 matings yielded frequencies of 456 red, 869 bronze, 404 sooty and 540 black. If these genes are nonallelic and independent in their transmission, the backcross to sooty would be expected to produce progeny in the ratio of 1 red:1 bronze:2 sooty, provided the genotypes (*bbSs*) and (*bbss*) are indistinguishable from black and individuals of the genotype (*Bbss*) are identical with sooty. Under these conditions the backcross to black would yield one bronze to one black, while the F_2 would have an expected frequency of 3 red:6 bronze:3 sooty:4 black. Chi-square tests revealed none of the observed results to deviate significantly from the expected. These findings indicate that the mutants (*b*) and (*s*) belong to different linkage groups. Additional data indicate neither black nor sooty to be linked to a third autosomal mutant (pearl) which affects the eye pigmentation in *Tribolium castaneum*. (Work supported by a grant from the National Science Foundation.)

BAUMILLER, R. C., S. J., and I. H. HERSKOWITZ, Saint Louis University, St. Louis, Mo.: *The relation between phenotypic detriment of heterozygous mutations and genetic load in Drosophila.*—*D. melanogaster* containing different loads of mutation were obtained by not irradiating a wild type strain (C) and by X-raying inseminated females of a sibling strain (L) with 1000r in each of 50 generations. In the next two generations neither strain was irradiated. To study the phenotypic detriment of adding to the two strains equal numbers of mutations in heterozygous condition, each strain was then divided into untreated (U) and X-rayed (X) sublines; in the latter sublines, males were given 3000r then mated with sibling virgins. From inseminated females of each subline (CU, CX, LU, LX) eggs were collected at half-hour intervals, and each hour, be-

ginning with the 21st hour after oviposition, the number of larvae hatching were scored. Of all larvae, the percentages requiring 27 or more hours to hatch were: CU, 3.2 ± 0.3 (of 5712 larvae); CX, 7.0 ± 0.5 (of 2216 larvae); LU, 4.9 ± 0.2 (of 7958 larvae); LX, 13.9 ± 0.6 (of 2637 larvae). —The difference, of about 1.7%, between LU and CU represents the detrimental phenotypic expression of the genetic load accumulated from previous irradiations. Within each strain X was greater than U, confirming our earlier results on the phenotypic detriment of heterozygous X-ray-induced mutations (Drosophila Information Service, 33: 122. 1959). However, the difference, of about 6.9%, between LX and CX is significantly greater than the detriment due to the mutational load previously accumulated. This demonstrates that, under the conditions described, the phenotypic detriment of euploid or near-euploid mutations in heterozygous condition is enhanced in the presence of a genetic load. (Supported by a grant from the U.S. Atomic Energy Commission (Contract AT (11–1)–633).)

BENDER, M. A., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *X-ray-induced chromosome aberrations in the Chinese hamster in vivo.*—The discovery that "fibroblasts" grown *in vitro* have much higher chromosome aberration rates, both control and irradiated, than epithelioid cells (PUCK, Proc. Natl. Acad. Sci. U.S. 44: 772, 1958) has made it imperative that chromosome aberration rates be determined for mammalian cells *in vivo*. Preliminary studies were made with the monkey, Ateles, but for an extensive study the Chinese hamster, *Cricetulus griseus*, was selected.—Males were given whole-body doses of 25, 50, and 100r of X-rays. Femoral bone marrow preparations were made at various times after irradiation. No aberrations were found in a total of 281 mitoses from three control animals. Two hours after 100r the total breakage rate was 50%. It fell rapidly with time; at 30 hours it was only 1%. Aberrations of all the expected types were found, but two-hit aberrations did not appear until the sixth hour. At two hours, the coefficient of aberration production was 0.005/cell/r. This value agrees with our previous value of 0.003 for epithelioid cells of both simian and human origin *in vitro*. It disagrees with PUCK's value (about 0.02) for human "fibroblasts." The control aberration frequencies of 0% for bone marrow *in vivo*, about 1% for epithelioid cells *in vitro*, and about 25% for human "fibroblasts" *in vitro* support the conclusion that epithelioid cells have about the same sensitivity as a rapidly dividing tissue *in vivo*, whereas "fibroblasts" are much more sensitive.

BERT, GRACE R., (Introduced by H. L. CARSON), Washington University, St. Louis, Missouri: *Fixed heterozygosity and fitness in Drosophila melanogaster populations under strong natural selection.*—Six replicate vial populations of *se ss ro* ("sesro") stock were brought to equilibrium size under carefully controlled laboratory conditions. Single males heterozygous for the sesro stock chromosomes and for the Curly and Payne inversions on chromosomes II and III respectively were added to two populations; crossing over between the introduced chromosomes and the sesro chromosomes was thus prohibited. Single males heterozygous for sesro chromosomes and Oregon-R chromosomes were added singly to two other populations; free crossing over was permitted in these populations. Two populations were retained as controls without introductions. Within 16 weeks the Curly inversions were eliminated from the inversion populations leaving the second chromosome homozygous for the wild chromosome from the sesro stock, whereas the Payne inversions became balanced. Fitness of the three types of populations was measured weekly by biomass and number of individuals. Results show the Payne populations to be intermediate between the freely recombining populations and the controls. The non recombining third chromosome, alone, therefore appears to contribute significantly to increased fitness presumably through simple heterosis, without recombination. Aspects of fitness which included larval survival under crowded *versus* optimal conditions and weekly turnover of adults under crowding were compared in these populations. Results show a rapid adult turnover in the sesro controls as compared to a slower turnover in all four experimental populations, whereas survival from egg to adult is higher in the sesro populations.

BIANCHI, A., Plant Genetics Institute of the Catholic University, Piacenza, Italy: *The surface of the leaf in normal and glossy seedlings.*—The wettability of the leaf surface in maize seedlings varies greatly according the genotype, *Gl* and *gl* mainly. Electron microscopy has made it possible to resolve the fine structure of the *Gl* surface as compared with those of seedlings homozygous for gl_1, gl_2, gl_3, and gl^H. The normal surface shows minute projections from the cuticle, projections that are almost absent, or very minute, in the glossy surface of young seedlings. This holds from the first to the fifth leaf for gl_1; gl_2, and gl_3 seedlings as well as for the third and the fourth leaf for gl^H. When the normal leaf is washed in xylene, the blade surface appears very similar to the untreated *gl* surface.

BIANCHI, A., G. MARIANI, and P. UBERTI, Institute of Genetics, University of Milan, Italy: *Endosperm and seedling mutations induced in maize, following X-irradiation and diepoxybutane treatment of mature pollen.*—Plants homozygous for $a_1sh_2lg_1$ and *pr* were fertilized with pollen $A_1Sh_2Lg_1Pr$ treated with diepoxybutane and or 1–2 kr.—Diepoxybutane turned out a good inducer of mutant endosperm sectors, confirming the results of KREIZINGER, who used genetic markers in chromosome 9. However, diepoxybutane had negligible effect on the F_1 generation, again as found by KREIZINGER—There may exist an interaction between the action of diepoxybutane and X-rays dose. The latter may or may not give different results when the kvp is 30 or 230. Furthermore, the following conclusions could be drawn: *Pr* is lost about one third less frequently than A_1Sh_2 (the latter two being, usually, lost together); the frequency at which they are lost is an exponential function of X-ray dose; the sector losses, however, in the material X-rayed only, increases almost linearly with the dose; the material treated with diepoxybutane and X-rays does not show concomitant variations for sectorial losses at the various X-ray doses.—The frequency of induced germless is exponentially related to X-ray dose while the remaining dominant lethals appear as an almost linear function of the doses. The frequency of dominant lethals in a_1sh_2 kernels is at least twice as high as in A_1Sh_2Pr kernels.

BLUMEL, JOHANNA, Department of Surgery, Division of Orthopedics, and Tissue Culture Laboratory, University of Texas Medical Branch, Galveston, Texas: *The effects of N-orotyl-N'N'-diethyl-ethylenediamine HCl on cells grown in tissue culture.*—With the benefit of time-lapse cinematography the effects of N-orotyl-N'N'-diethyl-ethylenediamine HCl on cells grown in tissue culture were investigated. Five day old cultures of the Fernandes Amnion strain, grown in Rose chambers containing Eagle's medium, were treated with Eagle's medium containing 5mg/ml of the chemical. The injurious effects of the chemical were dramatically illustrated after two days. At that time the medium containing the noxious agent was removed and one half of the chambers were treated with plain Eagle's medium and the remaining chambers were treated with Eagle's medium containing 2.5mg/ml thymine. The thymine treated cells recovered very rapidly after the addition of this chemical, but after the initial recovery the thymine itself seemed to exert some toxic effect. In toxicity tests 2.5mg/ml thymine was on the borderline of toxicity to the cells. The recovery of the cells was more gradual in the chambers containing the plain Eagle's medium. The action of N-orotyl-N'N'-diethyl-ethylenediamine HCl, a possible orotic acid antagonist, will be discussed.—The N-orotyl-N'N'-diethyl-ethylenediamine HCl was kindly supplied by DR. JOHN KOK, University of Amsterdam, Holland.—(This work was supported in part by the U. S. Army Medical Research and Development Command, Department of the Army, under Contract No. DA–49–193MD–2087, administered by DR. C. M. POMERAT.)

BOSE, SMRITIMOY, Blandy Experimental Farm, University of Virginia, Boyce, Virginia: *Cytological studies in Setcreasea.*—Cytological studies on root tips made for the first time in *Setcreasea purpurea* revealed a somatic number of 12. In *S. brevifolia* a new aneuploid number of $2n = 25$ was observed. Three major types of chromosomes were recognized in the karyotypes of both taxa. These were: A—median centromere, B—submedian centromere and C—submedian centromere with satellite on the shorter arm. Type C was a new type of chromosome found for the genus. The extra chromosome in *S. brevifolia* was one of the subtypes of type B. Meiosis in *S. purpurea*

was regular with six bivalents. The chiasma frequency per bivalent was 2.7. In pollen grain mitosis six chromosomes could be distinguished.—In *S. brevifolia*, univalents, bivalents, trivalents and quadrivalents were common in meiosis. The chiasma frequency per chromosome was 0.9. The chromosome distribution at anaphase I was 12–13 in 89 percent of the cases; the remaining were 11–14 in seven percent and 10–13(?) in two percent of the cases. Eight percent of the cells in anaphase I had chromatid bridges with fragments. Chromosome counts from pollen grain mitosis showed seven percent with 11, 32 percent with 12, 44 percent with 13, one percent with 14, and 13 percent with 25 chromosomes. The pollen grains varied markedly in size. Many cases were observed of abnormal cytokinesis in pollen grain formation. The percentage of normal appearing pollen was 77 percent.

Brady, Patricia, Rei Kato, and George Yerganian. The Children's Cancer Research Foundation and Harvard Medical School, Boston, Massachusetts: *Mitotically stable in vitro cell lines of the Chinese hamster.*—A number of single cell clonal derivatives from normal and SE Polyoma virus-induced sarcomas have remained stable with respect to chromosome number and the presence of distinct marker chromosomes. The majority of initial clonings failed to yield constant types. Among the more interesting clones are A31 and A45, which are hypodiploid with 19–21 chromosomes and only one X chromosome. Clone B39f is quasidiploid (XO) and grows more rapidly at 42°C than at the normally prescribed 37°C.—Subclones of Clones 2 and 3 of Bender have yielded stable, near-diploid, -triploid, and -tetraploid sublines necessary for radiation trials. They have low spontaneous chromosome aberration rates and adequate plating efficiencies. They appear to adapt readily to current spinner culture procedures for continuous maintenance in the log phase of proliferation.—An adult female fibroblast derivative is exceptional and has persisted for over one year in the classic diploid state. An increase in proliferative ability occurred after the 38th passage. Such lines are considered to be the result of chance occurrence and absence of events that lead to aberrancies. Inherent selection favors development of more rapidly proliferating types. Viral tumors have stem cell types which, unfortunately, are readily overwhelmed by rapidly proliferating derivatives which have shorter generation times and/or undergo mitosis immediately following trypsination and subculture. Stem cells can be isolated by the single cell procedures of Puck *et al.* as a means of regaining the primary tumor karyotype.

Braver, G., University of Oklahoma, Norman, Oklahoma: *The influence of an adjacent inversion breakpoint on unequal crossing over in the Bar region of Drosophila melanogaster.*—The reduced recovery of crossovers near breakpoints of inversion heterozygotes is probably due to mechanical interference with synaptic pairing at the points of change of linear homology. This could result in nonpairing and/or approximation of non-homologous loci in the regions immediately adjacent to the breakpoints. Both conditions, particularly the latter, might result in an increase in the ratio of unequal crossing over/total crossing over in the duplicated region of Bar flies. *In(1)AB* is a medium-sized inversion of the X chromosome, with its proximal breakpoint just distal to the Bar region. There is about 0.1% crossing over between Bar and the inversion. The offspring of females of genotypes *B car/f B Bx* and *In(1)AB, B car/f B Bx* are being screened for round-eyed individuals, these the product of unequal crossing over in the Bar region. In some crosses the autosomes are normal, in others they are heterozygous for autosomal inversions in order to increase exchange in the X chromosome. Results of this investigation will be presented and discussed. (This work was supported in part by a grant from the Faculty Research Committee of the University of Oklahoma.)

Bray, David F.,[1] A. E. Bell, and S. C. King, Population Genetics Institute, Purdue University, Lafayette, Ind.: *The importance of genotype × environment interaction with reference to control populations.*—Fifteen methods of maintaining control populations have been studied over eight generations using the flour beetle, *Tribolium castaneum.* Populations were reproduced each generation from 50 males and 50 females. The methods were compared as to their ability to establish the level of the environment with respect to the base population and to separate en-

vironmental and genetic effects in a selected line. It was demonstrated that a foundation stock of these beetles produced pupae which weighed about 10% more when grown in 70% relative humidity than when grown in 40% r.h. These two environments were applied to generations in an alternating manner. Thus, it was possible to predict the expected weight in any given generation.—Inbred lines derived from a separate source of stock reacted differently to the environments than did the foundation stock from which the selected lines were drawn.—No differences among the methods of maintaining the base population were observed.—Response to selection in both directions over eight generations was symmetrical. Within environment regressions indicated the development of a genotype × environment interaction. Selection in the dry environment was more effective than selection in the wet environment.—It is concluded that a control line must be closely related to the selected line in origin and time in order for its reactions to environmental shifts to be similar in nature to the selected line. It is unnecessary to place restrictions on the method of mating control stock base populations provided that at least 50 males and 50 females are used. ([1] Now at The University of British Columbia, Vancouver 8, Canada.)

Brewbaker, James L., and George C. Emery, Brookhaven National Laboratory, Upton, New York: *Incompatibility allele mutations.*—Mutants of incompatibility alleles in petunia have been sought among roughly 12 million pollen grains in five homozygous and ten heterozygous genotypes, and two spontaneous and 63 induced "mutants" genotypically identified. Spontaneous mutation rates based on seed recovered from incompatible matings of homozygous clones averaged 4.9 per million pollen grains, while heterozygotes averaged 67.9 per million. Premeiotic irradiations of 1000r increased these rates in homozygotes to 26.0 per million and impressively in heterozygotes to 1980.0 per million (1168 per million at 500r).—Among the 65 "mutants" were 21 with altered incompatibility behavior, of which 17 were self-fertile due to loss of pollen activity. All of the self-fertile mutants analyzed were the result of addition rather than deletion of an *S* locus in the genome. Such addition permits the inclusion of two different *S* genes in a single pollen grain, a condition leading to the breakdown of incompatibility by "competition" interaction (see Emery, Brewbaker and Shapiro). This competition combination was achieved in at least five plants by the addition of an *S* gene bearing centric fragment to the genome.—Five other "mutant" plants were totally sterile, and four female sterile and many "mutant" seed were nonviable. All of the 18 plants from homozygote parents and 19 of the 47 from heterozygotes were of nonmutant genotypes expected as a result of chance weakening of the incompatibility barrier. No new alleles were obtained. The data cast some question on current theories of *S* gene bipartite structure, revertible gene mutation and time of *S* gene action in microsporogenesis. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

Brooks, Margaret H., Oklahoma State University, Stillwater, Okla.: *A study of the mode of gene action in cytoplasmic male sterility: semiquantitative analyses of the free amino acids in anthers of fertile and cytoplasmic male sterile Sorghum.*—A study is currently underway to attempt to establish the biochemical basis for cytoplasmic male sterility and to better understand the nuclear-cytoplasmic interaction involved. Several varieties of *Sorghum vulgare* obtainable both as fertile lines and as sterile isogenic lines are being comparatively studied. One approach is through paper chromatography of anther extracts to determine what, if any, metabolites might accumulate as the result presumably of altered enzyme action. So far as the microspore-prepollen stage, which is terminal for the sterile lines, is concerned, no qualitative differences have been detected for the following free amino acids: aspartic acid, glutamic acid, serine, threonine, and alanine. Semiquantitative studies reveal that in all cases the concentration of these free amino acids is higher in the fertile anthers than in the steriles when compared on a per anther basis. This might be expected since the average dry weight of the fertile anther is 1.8 to 2.8 times greater, depending upon the variety. However on a unit of dry weight basis, using one-dimensional chromatography, in two varieties (Martin and Combine-60) alanine appears in higher concentration in the fertiles, whereas both serine and glutamic acid show a higher concentration

in the steriles. In a third variety (Redlan) these three amino acids show a higher concentration in the fertiles. Using a two-dimensional method the "alanine spot" separates into alanine and another unidentified amino acid. Threonine and aspartic acid are absent or present in only small amounts with the methods used. While pointing up possible varietal differences, at this time these results do not appear to have any obvious relationship to sterility as such, and further analyses are underway. Analyses of all the common amino acids will be followed by analyses of nucleic acids, nucleotides, purines and pyrimidines, sugars and sugar phosphates and all studies will be repeated for the earlier stages of meiosis. Reports will be forthcoming on a comparison of these results with those for fertility restorer materials.

BROSSEAU, GEORGE E., JR., State University of Iowa, Iowa City, Iowa: *V-type position effects influencing the action of the Bar locus in Drosophila.*—Five derivatives of B^SY, recovered in an irradiation experiment, were found to exhibit variegation for Bar (B^{SV}Y). The genotype X/B^{SV}Y has a normal eye, while XY/B^{SV}Y has a narrow Bar eye, XY^L or XY^S/B^{SV}Y has an eye of intermediate size and shape. The Y chromosome does not modify the Bar phenotype of either B^SY or T(1:4)B^S. Minute (2)S10 and Enhancer-variegation 7, both strong enhancers of variegation, shift the phenotype of B^{SV}Y toward normal. These observations indicate that the *V*-type position effect is acting to suppress the *S*-type position effect responsible for the B^S phenotype causing a shift in the phenotype toward normal. Suppression of the *V*-type position effect with the Y chromosome allows the *S*-type position effect to act producing the Bar phenotype. In these cases, the action of the Bar locus depends upon an interaction between an *S*-type and a *V*-type position effect. (Work supported in part by research grant No. RG6508 from the National Institutes of Health, U.S. Public Health Service.)

BROWN, WM. P., and A. E. BELL, Purdue University, Lafayette, Indiana: *Alternative methods of selection applied to plateaued populations of D. melanogaster.*—Two plateaued populations of *D. melanogaster* which had been subjected to long-term selection for increased egg number constituted the genetic material. Intrapopulation analyses indicated that additive genetic variance for egg number was nearly nonexistent while nonadditive variance remained large. From interpopulation analyses the reverse was true, i.e., heritability was estimated at 0.19 ± 0.06, and nonadditive variance was small. The additive genetic variance in the interpopulation crossbreds represented new variance which was created by crossing the populations and had not previously been directly subjected to selection.—Four methods of selection were evaluated. The experimental design provided for the simultaneous testing of 200 individuals for egg number per generation in the first three methods with a selection intensity of 20 percent. Mass selection for high individual egg number was continued in each of the plateaued populations.—In the first method, mass selection was applied to a new closed population formed by crossing the two plateaued populations. An approximate 15 percent increase in egg production was achieved over the controls. Reciprocal recurrent selection between the two base populations produced a gain similar to that in mass selection accompanied by a decline in the level of egg production in the two purebred populations. The third method, a modification of reciprocal recurrent selection, gave an approximate 27 percent increase. A slightly greater decline in the purebreds was noted in this method. Inbreeding and hybridization was practiced between isogenic lines from each base population.—Evidence was obtained which indicated a negative genetic correlation between egg production in the crossbreds and purebreds. Terminal tests were conducted to judge the relative merits of the methods and also to compare them with the performance of the base populations. (Supported by a grant from the National Science Foundation.)

BROWNING, LUOLIN S., and EDGAR ALTENBURG, Rice University, Houston, Texas: *The relatively high rate of unequal crossing over between Brownex and white in Drosophila.*—In experiments with unattached-X Drosophila females heterozygous for Brownex and apricot (w^{Bwx} +/ + w^a), the regular crossover classes among the sons are normal-appearing reds (+ +) and presumably the double mutant w^{Bwx} w^a, which is phenotypically apricot (as indicated by attached-

X studies). Females heterozygous for these two complementary crossover classes would be red and would be recovered simultaneously in attached-X stock. In addition to the regular crossover classes, unattached-X females produce some white sons. JUDD suggests these whites are produced by unequal crossing over and are deficiencies. The complementary unequal crossover product would be the double mutant chromosome, Brownex-apricot. Attempts to recover whites from reds thrown by Brownex over apricot in attached-X stock were unsuccessful because an attached-X female having the two complementary products of unequal crossing over would be white-deficiency/w^{Bwx} w^a, and would probably not be red but apricot and thus phenotypically indistinguishable from the apricots produced by diagonal crossing over by a *trans* mother. In the present experiments with unattached-X's, a high proportion of the crossovers were whites, as compared with the reds (the only distinguishable product of regular crossing over in unattached-X females). Thus, among 252,000 males produced by unattached-X Brownex over apricot females, there were 18 whites and 13 reds. If, therefore, the whites are the product of unequal crossing over (and the reds of regular), then unequal crossing over between Brownex and apricot is at least as frequent as regular. In the present case, unequal crossing over occurs preferentially in one direction: 15 of the 18 whites were y^2, three were non-y^2 from y^2 $(+ w^a)/y+$ $(w^{Bwx} +)$ mothers. The high rate of unequal crossing over between Brownex and apricot might indicate that the white region represents, at least in part, a duplication. (This work has been supported by a grant from the National Institutes of Health, United States Public Health Service.)

CARLSON, E. A., Queen's University, Kingston, Ontario, Canada: *Is complementation mapping of complex loci valid for Drosophila?*—With recent advances in our knowledge of gene structure and function, there has occasionally been a tendency to assume that the successful techniques used for such studies in one organism are valid for all or most organisms. Historically the first attempt to use complementation for mapping a gene region was carried out by DUBININ, SEREBROVSKY, AGOL and others for the achaete-scute region of *D. melanogaster* during the early 1930's. This was shown to be inaccurate by several independent techniques and experiments performed by CHILD, by STURTEVANT, and by MULLER. In the more recent studies of complementation in microorganisms, this process has been used to define mutually exclusive units (separate genes) as well as functionally differentiated regions of a complex locus. Studies of several complex loci in Drosophila, especially the dumpy series, present serious difficulties for the use of complementation as a process for mapping complex loci. Thus, recombination tests directly provide a sequence: *o–lv–ov–v* for the four rightmost members of the dumpy series, where *o*, *l*, and *v* represent, respectively, oblique wings, lethality, and thoracic vortices. The combination *o/lv* is wild type; but *o/ov* is oblique and *o/v* is wild type. These show that the map distances for this series cannot be determined by the complementary relations of these mutants. Attempts at complementation mapping in Drosophila must be accompanied by detailed recombinational analysis if inaccuracies are to be avoided or if the series is to be considered pseudoallelic.

CARSON, H. L., Washington University, St. Louis, Missouri: *Survival of newly-induced chromosome aberrations in experimental populations of Drosophila melanogaster:*—Three genetically different kinds of populations were maintained under strong natural selection; two of these are inbreds: (1) homozygous *se ss k* e^s *ro* ("sesro"); (2) wild type Oregon-R; (3) is outbred, having originated from an Oregon-R base population into which one haploid set of autosomes from sesro was added. After more than three years, this population still carries all five of the sesro genes in balanced condition. To date, two replicates of no. 1 have received 58,000r of X-rays each at the rate of 1000r to the adults/wk; one population of type no. 2 and one of type no. 3 have each received 37,000r. These populations maintain equilibrated sizes roughly two-thirds of the size of the corresponding nonirradiated control populations. Salivary gland chromosome examinations have been made of F_1 larvae from newly hatched pairs or single flies taken at random from the populations. Each fly tested hatched in the population under strong natural selection. Of 1103 chromosomes tested from irradiated inbred populations (nos. 1 and 2) over a period of a year and a half, none showed any visible aberration. Of 457 chromosomes tested from the outbred popula-

tion (no. 3), however, nine individually different aberrations have been observed, four inversions in 3R, three inversions in X and two translocations. Most aberrations appeared only once in single individuals or in two individuals which were apparently sibs. Of particular interest is one complex aberration which, at the time of writing, has been recovered in five different flies which hatched from the population over a period of about a month. This aberration consists of two independent inversions in coupling in the X chromosome (X-2, X-3); it has apparently survived under natural selection for at least two generations.

CHAI, C. K., and MORGAN S. M. CHIANG, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *Skin transplantation in mice of two fairly advanced inbred strains.*—Skin transplantation has been made between sibs and nonsibs in the Large strain and between sibs in the Small strain of mice. Both these strains of mice were developed by selection in the Large for large, and in the Small for small, body size for many generations and followed by inbreeding of brother-sister mating (for detailed breeding history, see CHAI, 1956, Genetics 41:157). In the Large strain, mice from the 10th to the 16th generations were used. They were all descendants from a common pair of ancestors in the ninth generation. A total number of 209 grafts have been observed for more than two months at the time when the report was written. The percentage of survival grafts made between nonsibs was 25.4, and between sibs 50.7. The distribution for the percentage of survival of sib grafts in the different generations was 35.3, 47.2, 54.8 and 85.7 for generations of 13, 14, 15 and 16, respectively. A total number of 30 sib grafts was made in the Small mice at 24th–26th generations of inbreeding. The average percentage of survival grafts was 83.3. Inbreeding effect on homozygosity for histocompatibility genes and the approximate number of loci involved will be discussed.

CHU, ERNEST H. Y., and VALERIO MONESI, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *Analysis of X-ray induced chromosome aberrations in mouse somatic cells in vitro.*—The present study was undertaken in order to have a comparison with the previous observations of X-ray effects on human chromosomes and to correlate with other cytogenetic effects of radiations in the mouse. Embryonic skin fibroblast-like cells derived by trypsin-dissociation from 15–18 day old (101 ♀ × C3H ♂) F_1 fetuses were grown in culture. Rapid cell proliferation occurs within a day following initial explantation and in the subsequent two or three subcultures. Cells were grown directly on cover slips in depression test tubes (Leighton tubes) for 24 hours and X-irradiated with doses of 25, 50 and 100r, at an intensity of 100r/min. Cytological examination of chromosomes was made at 20 and 50 hours after irradiation, utilizing hypotonic-saline treated, colchicine-blocked metaphases. A euploid chromosome number ($2n = 40$) was found in a great majority of cells, and an idiogram of the mitotic karyotype has been constructed. Four autosomal pairs in addition to the Y chromosome are morphologically distinct and can be individually identified. Nevertheless, the general uniform appearance of the V-shaped chromosomes in the complement also facilitates detection and analysis of aberrations. In general, the majority of the various types of chromatid and chromosome aberrations have been detected. Only chromatid types were found at 20 hours after irradiation; the chromosome types were overwhelmingly predominant at 50 hours. Quantitative determinations for both chromatid and chromosome types of aberrations show a dose-effect relationship compatible with observations on other classical materials, but indicate a much greater radiosensitivity in this system, similar, perhaps, to the human somatic cells under comparable experimental conditions.

COE, E. H., U. S. Department of Agriculture and University of Missouri, Columbia, Mo.: *The pattern of origination of conversion-type inheritance at the B locus in maize.*—The initial change from orthodox to conversion-type inheritance of plant color occurs as a random event with varying probability. *B′* individuals appear in unstable *BB* lines at frequencies ranging between 0% (in progenies as large as 167) and 100% (in progenies as large as 45). The instability involved appears to be transmitted to progenies as either the same or greater instability, probably not less. "Ear maps" show that *B′* does not occur in sectors but arises in separate kernels as a consequence

of random independent events. The mutational event is, thus, placed in time between the initiation of ovule primordia and the germination of the kernel, suggesting that meiosis is responsible since it is the most likely stage in this period at which the event might be expected to occur. If the event is restricted to meiosis it would appear that its mechanism is chromosomal or genic.

CONNEALLY, P. M., N. E. MORTON, J. R. PATEL and W. H. STONE, University of Wisconsin, Madison, Wis.: *Multiple alleles at the J locus in cattle.* Analyses were carried out on serum scores (inhibition) of cattle classed as J^s (J in the serum but not detectable by ordinary tests on the cells), J^{cs} (J in the serum and detectable by ordinary tests on the cells) and j^a (no J in serum or on cells) to determine if the variation in the amount of J substance in the serum and on the cells could be due to a series of multiple alleles with different quantitative effects. Further, an attempt was made to determine how many alleles might be operating. A variety of statistical tests was performed. BARTLETT's discriminant function was used to weigh the dilution scores in the hope that it might afford better discrimination among progenies (groups of J^{cs}/j^a or J^s/j^a having the same J allele by descent) than equal weights. Analysis of variance within various progeny relationships was performed to determine the components of variation (modifiers, environment and effect of j^a allele). FISHER's discriminant was also used, but it yielded poorer discrimination than that of BARTLETT's. Scores of each group, J^s and J^{cs} animals, were tested for bimodality and each was shown to exhibit four modes suggesting a minimum of four alleles within each group. The results provided strong evidence for multiple alleles; however, it was not possible to propose with any degree of certainty the number of alleles operating. Genetic modifiers and interaction of the j^a alleles were shown to have negligible, if any, effect on the variation within the J^s and J^{cs} alleles. The effect of age on serum score was significant for both J^s and J^{cs}; the effect of heterozygosity with j^a on serum score was nonsignificant in both cases.

COTTER, WILLIAM B., JR., Department of Anatomy, Medical College of South Carolina, 16 Lucas St., Charleston, South Carolina: *A new developmental mutant in Ephestia kuhniella affecting antennal morphology.* In the Qy strain of *Ephestia kuhniella*, an exceptional male was found with extremely short antennae. This male was mated to a sister and the new strain, Qysa, was established. Preliminary analysis of this mutant strain showed that the over-all length of the antennae was 3.62 ± 0.47 mm, while that of a control stock, aaO, was half again as large, 5.12 ± 0.33 mm. In addition, the number of antennal segments was reduced slightly in the mutants (33–48) when compared to the controls (49–53). A modification of the normal long rectangular, distal segments of the antennae was seen as a homonomous series of short rectangular or square segments, similar to those normally found in the proximal portion. Occasionally, the terminal segments appeared ovoid, with their long axes perpendicular to the long axis of the antenna. Other characteristics of the short antennae were deformation of the filamentous appearance by curves and kinks along their length, a high degree of transparency in the most distal segments, and increased fragility of the antennae when handled in measurement. Supporting, through inference, the last characteristic were the relatively greater numbers of *sa/sa* animals with broken antennae as a direct consequence of normal behavior in culture. Concurrent measurements of proboscis and eye width showed that the latter varied with the size of the total animal, and was independent of antennal length. The length of the proboscis showed a correlated shortening as the antennae were shortened, but without any gross malformation detectable. The above data are consistent with the hypothesis that the genes for short antennae interfere with the normal biosynthesis of the chitin of the exoskeleton. The gene *sa* is inherited as a simple autosomal recessive, the F_1 being all long antennaed. Segregation in the F_2 is complete, with only rare individuals intermediate between parental classes. Studies concerning modifiers and linkage relationship are now in progress.

CRUMPACKER, D. W., and R. W. ALLARD, Colorado State University and University of California, Davis, California: *A diallel cross analysis of heading date in wheat.*—The nature of a quantitative genetic system which governs heading date in a set of ten spring wheat varieties was

investigated. The parents chosen are the spring wheats which have been grown commercially, at one time or another, in California. Experimental materials consisted of all possible F_1 hybrids among the ten parental varieties. Diallel cross nurseries were grown in three different years at Davis, California.—Investigation of the assumptions upon which the diallel analysis was based indicated: (1) that there were statistically significant, but trivial, interactions of genotype with microenvironmental differences within years and (2) that nonallelic gene interaction (epistasis) was a minor, but statistically significant, feature of the genetic system. Neither of these disturbances appeared to be a source of serious bias.—The analysis suggested that the average degree of dominance in the system was partial and toward earliness. Cases of *apparent* partial dominance for lateness occurred in certain crosses. The dominance effects of different genes were unequal. There appeared to be an excess of recessive, as well as late, gene effects among the parents. The parents could be placed in one of three groups according to their levels of dominance: highly dominant, moderately recessive, and highly recessive.—The limits of selection within the system for genes *with dominance effects* are probably already represented by certain of the parental genotypes. The exact nature of the epistatic effects of the system was not determined.

Daly, K., Brookhaven National Laboratory, Upton, N. Y.: *The induction of quantitative variability by γ radiation in Arabidopsis thaliana.*—In a study of radiation-induced variability in *Arabidopsis thaliana* (L.) Heynh. (Cruciferae), changes in flowering time have been investigated. Seeds of the race 'Estland', previously stored over dry $CaCl_2$ at room temperature, were exposed to 50, 100, and 150 kr of Co^{60} γ radiation at a dose rate of 410 kr/hr. Seeds were immediately hydrated for one hour and then cultured aseptically on nutrient agar to maturity. Observations were collected on the date of flowering in first, second, and third generations following seed irradiation and transformed to common logarithms for analysis.—In the first generation, G_1, the means of all irradiated populations were shifted toward later flowering and variation was increased. A second generation, G_2, was cultured from a random sample of seed produced by the G_1 generation from self-pollination. In the three G_2 populations derived from 50, 100, and 150 kr exposures, the variance was significantly greater than in the nonirradiated population. The mean flowering date in each of these populations did not differ significantly from that of the nonirradiated population, 24.35 days. The within-plot variance in each irradiated population was partitioned into components associated with genetic and environmental effects. The regression of genetic variance on dose was not found to deviate significantly from linearity ($r = .99$).—From the G_2 generation, selections were made in each population for early and late flowering types, applying a selection pressure approximating 10%. The following generation, G_3, consisted of progeny tests of these selected individuals, comprising a total of 80 lines. Among the lines selected for earlier flowering in each of the three G_2 populations, 20–50% were found to be significantly earlier than the nonirradiated, selected population. However, the response to selection was not equally effective for late flowering; none of the late flowering selections were found to differ significantly from the nonirradiated, selected population. (This investigation was carried out during the tenure of a Postdoctoral Fellowship (CF-8248) from the National Cancer Institute, United States Public Health Service, while at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

Davidson, D., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *Polyamines and chromosome condensation.*—Polyamines occur in sperm and in association with viral DNA. The action of the diamines, putrescine and cadaverine, and of the tetramine, spermine, on cells undergoing mitosis will be described from studies in which plant roots were used. All the stages of mitosis can be affected within a six-hour period of treatment with 0.5 mM solutions. At anaphase-telophase, chromosome uncoiling and the formation of a normal, spherical nucleus is prevented; the nuclei become palmate. At metaphase the chromosomes condense and stain heavily with Feulgen, but instead of forming the diploid number of individual chromosomes, a single, amorphous structure is formed. Treatments longer than six hours suppress all mitotic activity.—The effects of the three substances used indicate that polyamines and mitotic chromosomes form

associations not reversible by the cell within the normal duration of the period of late anaphase and early telophase changes. Also it seems that polyamines, in the concentrations used here, are not under cell control in their action on prophase and metaphase chromosomes. They act indiscriminately during these stages, leading to fusion of chromosomes. Since polyamines appear to interfere with the processes of chromosome coiling and uncoiling, it may be that naturally occurring polyamines are involved in the mechanisms of spiralization that result in chromosome condensation.

De Marinis, F., and Fred E. Sheibley, Fenn College, Cleveland, Ohio: *Preliminary tests with some synthetic amides on the action of Bar eye Drosophila.*—Kaji (1955, 1956) has shown that the amide linkage, –CO·NH–, increases the facet number in Bar eye Drosophila, and that acetamide, exerting its greatest effect in larvae 60–80 hours after hatching, changes a genotypic Bar eye to a phenotypic wild type. These results are largely confirmed by our recent experiments. In addition, when acetamide is given to later stages, 80–90 hours after hatching but just before pupation, it affects the wings and body, but not significantly the eye.—Thioacetamide is highly reactive and upsets major physiological reactions in development. Larvae, 51 hours after hatching, were placed on food containing 2% of thioacetamide for one half hour and produced highly distorted adults many of which had black, tumor-like structures in the body.—Preliminary trials were conducted with a series of quinazolines, compounds which contain the amide linkage in a cyclic structure rather than in an open chain. 4-Quinazolone, benzoylene urea, 2-ethoxy-4-quinazolone, and 1-ethylbenzoylene urea were fed during the critical larval period and did not show any significant activity on facet number. The tests indicate that the amide linkage in a ring is not as effective as it is in a linear structure. 4-Quinazolone, fed to young larvae 20–30 hours after hatching, prolonged their larval period by more than ten days and prevented metamorphosis.

Desborough, S., E. E. Shult, and C. C. Lindegren, Southern Illinois University, Carbondale, Ill.: *Interference patterns in a Saccharomyces family.*—From the family Y-1, 140 asci were dissected and tested for 15 Mendelian segregating phenotypes. Four new loci, not previously described, were made heterozygous in this hybrid. Of these, three were found to be linked with known loci. The loci are ordered on Chromosome I as: *UR* – Centromere – *TZ* – *AG* – *IS* – *NI*. The family exhibited two unusual features of crossing over in this chromosome: (1) the appearance of strong negative chromatid interference in the portion distal to *TZ*, (2) an extreme excess of a single exchange localized in the segment centromere – *TZ*. It was shown that the former effect was genuine in the sense that it is not the result of faulty assignment of gene order or non-additivity due to quasi-linkage. It was also proved that the latter is true chromosomal interference, rather than the result of three-strand doubles. The localized exchanges in centromere – *TZ* do not appear to engage in the negative chromatid interference pattern described in (1). Another unusual feature of Chromosome I in this family is evidence of an inversion containing the *IS* locus.

Dubes, G. R., and E. A. Klingler, Jr., University of Kansas School of Medicine, Kansas City, Kansas: *Facilitation of infection with poliovirus "ribonucleic acid."*—Appropriate treatment of intact poliovirus with phenol converts it from an infectivity stable to ribonuclease to a ribonuclease-labile infectivity which is called infective poliovirus "ribonucleic acid" or "RNA". Much genetic interest attaches to this "RNA" since it appears that it carries the poliovirus genetic information. The plaque titer on normal monkey kidney tissue cultures of this "RNA" is only about one three-millionth of the plaque titer of the intact virus from which it was prepared. The natural mechanism for entering the cell is very probably lacking in this "RNA", and inability to enter may account for the very low plaque titer of "RNA" relative to intact poliovirus. We have provided the "RNA" with what appears to be an artificial entry mechanism and have so increased its plaque titer 1000-fold. This increase is obtained by mixing the "RNA" with a suspension of $CaHPO_4 \cdot 2H_2O$ or any one of several other poorly soluble compounds before inoculating it onto monkey kidney tissue cultures specifically deficient for calcium. Substances which will substitute

for $CaHPO_4 \cdot 2H_2O$ include dibasic and tribasic magnesium phosphates, calcium and magnesium carbonates, aluminum oxide, and magnesium fluoride. With tribasic magnesium phosphate adsorption of the "RNA" to the finely suspended phosphate particles was demonstrated. These compounds probably facilitate infection by serving as solid vehicles to carry the poliovirus "RNA" to the cell and there so dispose the "RNA" that the probability of its successful entry into the calcium-starved monkey cell is greatly increased. (Supported by the National Foundation.)

DUTTA, S. K., and E. D. GARBER, University of Chicago, Chicago, Ill.: *Heterokaryosis in Colletotrichum lagenarium.*—The imperfect fungus responsible for anthracnose of cucurbits, *Colletotrichum lagenarium* (Pass.) Ell. & Halst., is a wide-spread and important pathogen. Color and biochemical mutants were isolated after spores of two physiological races were irradiated with ultraviolet light. Heterokaryons involving nutritionally deficient mutants of the same or different races were easily produced and displayed a characteristic color and morphology, depending on the component strains, on complex and minimal media. Attempts to isolate spores with a diploid nucleus were unsuccessful. (This research has been supported by the U. S. Public Health Service and Abbott Memorial Fund, Univ. of Chicago.)

EDINGTON, C. W., J. L. EPLER, and J. D. REGAN, Department of Biological Sciences, Florida State University, Tallahassee, Florida: *The frequency-dose relation of Y chromosome suppressed recessive lethals in Drosophila.*—LINDSLEY and EDINGTON (1957) described a modified Muller-5 technique that would allow detection of sex-linked recessive lethals whose expression is suppressed or enhanced by the presence of a Y chromosome. In addition, those lethals normally detected in recessive lethal experiments are also recovered. Experiments using the above techniques have been conducted in this laboratory to determine the frequency-dose relation of total lethals (including the exceptional lethals described above) induced by X-rays. Evidence will be presented that may explain why nonlinear frequency-dose relations for sex-linked recessive lethals in Drosophila have usually not been detected. It is clear that total lethals induced by X-rays increase faster than expected on the basis of linearity and that exceptional lethals (presumed to be position effect lethals) increase as the 1.5 power of the dose. (This work supported by contract AT(40–1)2417 from the Atomic Energy Commission.)

EHLING, U. H., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *Strain differences in the reproductive performance of female mice after X-ray exposure.*—From 14 to 18 female mice of each of the strains, SeC, A. SeAb, NB, and dba, were irradiated with 50r (80r/min). Heads were shielded with lead. One day after exposure each female was caged separately with a nonirradiated C3H male for the duration of the experiment. Equal number of nonirradiated females were mated in the same way. Litters were recorded and killed at birth.—Fertility of females after exposure with 50r, expressed as percent of total production of the control group over a period of 130 days, was SeC (30%), A (36%), SeAb (40%), NB (52%), and dba (100%).—The results clearly demonstrate strain differences in reproductive performance following irradiation. Comparisons between species must take this intraspecific variation into consideration. It is noteworthy that the dba strain, which shows the highest relative fertility after irradiation, was a poor fourth in rank in productivity in the controls. Factors other than differential radiation sensitivity of oocytes may be involved. For example, as OAKBERG has suggested, strains may differ in the total number of oocytes present before irradiation. Experimental analysis of strain differences is now in progress. (This investigation was supported in part by a fellowship from the National Institutes of Health, Public Health Service.)

EISENSTARK, A., and RUTH VAN SICKLE, Kansas State University, Manhattan, Kansas: *Antigenic mutants of phage T3 of Escherichia coli.*—A previous report (EISENSTARK, MAALØE, and BIRCH-ANDERSEN, Bacteriological Proceedings, 1960, page 81) described variants of phage T3 that differ with regard to antigenic, morphological and host range properties. Antibodies against one of these phages, designated as T3B, are incapable of neutralizing another T3 strain, designated as

T3C. Anti-T3C sera, however, can inactivate both T3B and T3C. Serum blocking power tests show that T3C in fact contains two antigens: the one concerned in the inactivation of T3B plus an additional antigen produced by T3C alone. Also, the tail structure was found to be different in these two strains: T3C has a long flexible tail, in contrast to T3B which has a short snout.—In addition to these two phage variants, a minute plaque-forming phage, designated as T3M, has been found which contains still another neutralizable antigen. Neither anti-T3B sera nor anti-T3C sera can neutralize T3M. T3M is a mutant of T3C that occurs in a frequency of 10^{-6}. A number of T3M mutants have been isolated which differ from each other in a rather striking manner, particularly with respect to clarity of minute plaques and host range.—Recombination studies are in progress to determine whether the antigenic differences in these T3 phages are consequences of point mutations, deletions, or reverse mutations. Particularly, an attempt will be made to correlate the observed "structural gains" with genetic events. (This work supported by National Institutes of Health, Grant No. E-3139(A).)

EMERY, GEORGE C., J. L. BREWBAKER, and N. SHAPIRO, Brookhaven National Laboratory, Upton, N.Y., and Purdue University, W. Lafayette, Ind.: *Polyploidy and self incompatibility in Petunia.*—Diploid seed of five homozygous *S* allele (incompatibility) genotypes of *Petunia inflata* were colchicine treated and the corresponding five homozygous tetraploids recovered. These tetraploids were highly self incompatible, averaging 0.08 seeds per self-pollination, with the diploid homozygotes averaging 0.01 seeds per flower selfed. The ten heterozygous diploid genotypes obtained by crossing the five diploid homozygotes were similarly self incompatible (0.3 seeds per self pollination). However, all of the corresponding tetraploid heterozygotes were self-fertile, yielding an average of 106 seeds for each self-pollinated flower. Testcross data demonstrated that the self-fertility of all tetraploid heterozygotes was due to "competition interaction" between two different *S* alleles in heterogenic diploid pollen grains. Similar competition interaction has been observed comparatively rarely among tetraploid heterozygous genotypes of *Oenothera organensis*, *Trifolium repens*, and *Trifolium hybridum*.—Triploid heterozygous clones obtained by crossing tetraploid homozygotes as females to unrelated diploid homozygotes were fully self incompatible. Cytological analysis indicated that viable pollen produced by the triploids were almost exclusively haploid, thus eliminating the possibility of self-fertilization by heterogenic *S* allele pollen. Approximately 50 trisomics of *P. inflata* have been recovered from crosses of triploids as females with diploid homozygotes, and cytological identification of the chromosome bearing the *S* locus is in progress. Evidence has been obtained (see BREWBAKER and EMERY) that competition interaction accounts for a majority of *S* gene mutants recovered in petunia as a result of *S* gene duplication in the genome (e.g., addition of centric fragments). Studies of the *in vitro* behavior and physiological nature of the competition pollen tubes will be reported. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

ERK, FRANK C., State University College on Long Island, Oyster Bay, N. Y.: *A study of genetic control over segregation in a translocation heterozygote.*—A radiation-induced translocation between the second and third chromosomes in *Drosophila melanogaster* was marked with suitable genes to permit a study of the frequencies of both orthoploid and aneuploid gametes produced within the same strain over a number of successive generations. The breeding scheme was designed to guarantee the reappearance of the initial orthoploid chromosome types in certain flies in alternate generations; these flies were then mated to continue the strain. Eleven strains and substrains were maintained for 12 generations by means of this technique, in which only flies resulting from the union of orthoploid gametes were mated, and the percentages of orthoploid gametes formed ranged from 47.17 to 79.78 in single generations of the various strains. A statistical analysis of the results indicates that no significant genetic control over the ability of a strain to produce an increased percentage of orthoploid gametes had developed within the strains over this period of time, although one strain showed definite promise for future selection for a high percentage of orthoploidy.

FANTINI, A. A., and LINDSAY S. OLIVE, (Introduced by S. K. DUTTA), Department of Botany, Columbia University, N. Y.: *Genetics and antibiotic production of Emericellopsis species.*—Emericellopsis, a fungus belonging to the order Eurotiales, has both a perfect (cleistothecial) and an imperfect (cephalosporium) stage. By U.V. irradiation, a number of auxotrophic and morphological mutants were induced. These were combined in various sexual crosses, and the ascospores from single cleistothecia analyzed. Crossed cleistothecia occurred rarely (3%), but conclusive proof of meiotic recombination was obtained. Antibiotic yields of parentals and recombinants were then compared.—While prototrophic recombinants obtained from sexual crosses usually produced as much antibiotic as the original wild type strain, it was shown that other factors possibly linked to a morphological marker have an effect on the antibiotic yield. Recombinants obtained from a cross between a high producer and a low producer were intermediate in range.—Experiments designed to produce recombinants by a parasexual or mitotic process were generally inconclusive, but some evidence of recombination of genetic material was observed. No stable heterozygous diploids were observed.—The process of heterokaryon formation appears to be limited in frequency and extent. Antibiotic yields by heterokaryons synthesized from auxotrophs of low antibiotic-yielding ability usually approach the activity of the original wild type strain, but a decrease was observed with the dissociation of the heterokaryon.—Yields higher than those produced by the wild type were observed in only two instances: in one case in a U.V. induced mutant with somewhat restricted growth, and in another in what appears to be a somatic recombination within the mycelium with expression through a sexual stage.—The auxotrophs used in these experiments were found to be quite stable, and no revertants were observed in any of the conidial platings at concentrations of about 10^6 conidia/ml. (Supported by a fellowship from The Eli Lilly Company).

FOGEL, SEYMOUR, and DONALD D. HURST, Brooklyn College, Brooklyn N.Y.: *Coincidence relations between gene conversion and somatic recombination in Saccharomyces cerevisiae.*—In diploids heteroallelic for an adenine marker (ad_6) and containing a system for signalling conversional events, the coincidence relations between gene conversion and homozygosis among other unlinked markers was studied in U.V.-treated and control populations.—Among 87 instances of conversion at the ad_6 site, there were ten cases of coincident homozygosis for an unlinked uracil (*ur*) marker. That the conversional event is essentially nonreciprocal is indicated by the behavior of linked outside markers. However, a disturbance of normal centromeric behavior is indicated. Convertants frequently arise as sectored clones, and homozygosis via somatic recombination, a reciprocal process, for unlinked markers might be expected to be distributed by a conventional mitotic mechanism; in 8/10 cases of coincidence the newly arising convertant allele is associated with the recessive homozygote for *ur*. Moreover, the frequency of homozygosis for *ur* is approximately ten times greater among convertants than among nonconvertants with a given dosage of U.V. irradiation. (This work is supported by a grant from the National Institutes of Health.)

FOGEL, SEYMOUR, and DONALD D. HURST, Brooklyn College, Brooklyn, N.Y.: *Coincidence relations of somatic recombination in Saccharomyces cerevisiae.*—Utilizing the adenine (ad_x/ad_x Ad_x/ad_x) mutation signal system (red to white clone) coincidence of homozygosis in unselected, nonlinked markers was studied in ultraviolet and control populations.—The frequency of coincident events, i.e., simultaneous homozygosis at the Ad_x signal locus and other nonlinked loci was found to be at least tenfold greater than anticipated on the assumption of random independent events. For the majority of coincident cases, non-homologous chromosomes appear to be distributed in a normal fashion. However, in some instances a distinctly polarized segregation has been observed.—The high coincidence values along with directed segregations suggests the existence of an interchromosomal effect in somatic recombination, a process that is essentially reciprocal in character. (This work is supported by a grant from the National Institutes of Health.)

FORD, LEE, Pacific Lutheran College, Parkland, Washington: *A study of genetic and hematological aspects of the lethal gene d producing the silver collie pups.*—The homozygous recessive condition of the gene *d* produces a pup which is phenotypically "silver grey", which may be born dead, live for a few days, or may possibly live to four to six months of age. The silver pups seem to be lighter in bone and weight and more susceptible to bacterial infection than their sibs. The heterozygous carriers cannot be phenotypically distinguished from the homozygous normal pups and appear to be normal. Blood studies involving RBC and WBC counts, differential WBC counts, sedimentation, coagulation, hematocrit fragility and hemaglobin analysis were made of the greys and normal control dogs. The results indicated wide deviation from normal, becoming more so with increasing age of the dog and approach of death.

FORSTHOEFEL, PAULINUS F., University of Detroit, Detroit, Mich.: *Further studies on the effects of Strong's luxoid gene.*—Crosses of mice heterozygous for both STRONG's luxoid gene and GREEN's luxoid gene with normal C57BL/10 mice have shown that the two luxoid genes are not alleles and are not linked. Similar crosses of mice heterozygous for both STRONG's luxoid gene and CARTER's luxate gene with normal C57BL/10 mice have shown that these two genes are neither alleles nor linked.—STRONG's luxoid gene interacts with GREEN's luxoid gene in the double heterozygote to produce tibial hemimelia often accompanied with partial duplication of the reduced tibia and polydactyly of the forefeet. Matings of these double heterozygotes together have produced many F_2 offspring, among whom more extreme phenotypes appear. STRONG's luxoid gene interacts with CARTER's luxate gene to produce tibial hemimelia. Matings of these double heterozygotes together have also produced many F_2 offspring, some with more extreme phenotypes. Matings of mice doubly heterozygous for STRONG's luxoid gene and CARTER's luxate gene with mice heterozygous for GREEN's luxoid gene have produced mice whose distinctive phenotype indicates heterozygosity for all three genes.—The foregoing results show that STRONG's luxoid gene interacts with GREEN's luxoid and CARTER's luxate genes on the parts of the skeleton it affects in common with them, but not always in a simple additive manner, as shown by the strong tendency of the reduced tibia to be duplicated in mice heterozygous for both STRONG's and GREEN's luxoid genes. (Supported by Grant C-3613 from the National Institutes of Health.)

FUERST, ROBERT, Texas Woman's University, Denton, Texas: *Studies on the induction of paramorphism by some new compounds.*—Paramorphism in Neurospora is not a new phenomenon. L-sorbose has been extensively applied to induce colonial growth in mutation experiments in this organism. The genetic control of colonial mutants has also been thoroughly studied. Other agents tested, that induce colonial growth, were gammexane, tergitol-7 or sodium desoxycholate, duponol, *p*-hydroxybenzophenone azine, 6,8-diamino-2,3-dimethyl quinoxaline, and a number of thiophene derivatives. 2-(2-ethylhexyl)thiophene would cause the organism to grow in perfect circles that felt smooth and silky to the touch, while 2-(2-methylpropyl)thiophene and 2-heptylthiophene would feel coarse to the touch. Obviously the reasons for colonizing are different for these different compounds. Casein hydrolysate relieved the colonizing effect of these later two compounds. A high L-sucrose concentration of 10% to 20% would cause the mycelia to curl, except that in every case one or two hyphae in each colony would grow out in normal fashion. In all cases observed, the curling of the mycelia was in clockwise direction. At *p*H 3.0 Neurospora would still grow, but colonization would also occur. The effect of the compounds studied on some of the Neurospora mutants will be presented together with photo records of microscopic growth studies, as recorded with 35mm Ektachrome film.

FUNG, SUI-TONG, and JOHN W. GOWEN, Iowa State University, Ames, Iowa: *Role of autosome-IV in Drosophila melanogaster sex balance.*—Chromosomal determinations of the male and female intersexes from two selected triploid stocks (1959) show that their karyotypes consisted of two sets of X, three sets of II and III, with or without the Y, and having two, three, or

more IV chromosomes. Male and female intersexes differed from each other only in the number of IV chromosomes. Female intersexes possess two, three or more IV chromosomes while male intersexes generally have two. It appears that intersexuality in the triploids, aside from its dependence upon dosages of both the II and III chromosomes also is related to the number of IV chromosomes. The Drosophila X chromosomes have female tendencies while the autosomes are male-determining. Earlier we have reviewed other work showing that the third chromosome incorporates loci for male sex characteristics. Our present investigation shows the IV chromosome has greater female rather than male tendencies and is similar to the X and different from the male tendencies of the other autosomes. The recent work of KRIVSHENKO (1959) on the homology of chromosomal elements between *D. busckii* and *D. melanogaster* is of interest. His findings indicate that certain IV chromosome genes (Cell, shaven and cubitus interruptus) of *D. melanogaster* are homologous to the X chromosomal mutations of *D. busckii*. From evolutionary standpoint, the IV chromosome of *D. melanogaster* originally may have been derived from the X, and carried a portion of its female-determining tendencies with it. The female potencies of chromosome IV are rather weak requiring the 2X/3A balance to bring them out. (This work has received assistance from Contract Number AT(11–1) 107 from the Atomic Energy Commission.)

GARDNER, ELDON J., and WILLIAM D. BERSETH, Utah State University, Logan, Utah: *Population study of isoalleles associated with tumorous head in Drosophila melanogaster.*—The tumorous head phenotype is dependent on a basic third chromosome gene (*tu-3*), which behaves as a semidominant, and a sex-linked recessive (*tu-1*), which controls a maternal effect. The maternal effect was first indicated from a difference in the results of reciprocal crosses. An extract made from tumorous head females was later injected into the bodies of wild females and the maternal effect was transferred. The chemical nature of the effective substance is being investigated.—Isoalleles of the sex-linked gene are carried in several wild populations and three "wild" laboratory stocks (Stephenville, Crimea, and Oregon R.). Populations originating from flies with different initial combinations of these alleles have been cultured in population cages. In the population originating from a single pair of tumorous head flies the proportion of flies expressing the trait decreased from 89% to less than 10% in the first six months and became stabilized at a level between 5 and 10%. When the isoallele *tu-1*^{s} from the Stephenville stock was substituted for *tu-1* and a pair of flies was mated in another cage the expression remained fairly constant with a range from 63 to 76%. Another population originating from a heterozygous female carrying *tu-1* and *tu-1*^{s} also remained fairly constant in the expression of tumorous head but at a lower level, with a range from 21 to 34%. (Supported by the American Cancer Society and Damon Runyon Memorial Fund.)

GLASSMAN, EDWARD, Department of Genetics, City of Hope Medical Center, Duarte, California: *Genetic complementation between mutants of Drosophila melanogaster lacking xanthine dehydrogenase.*—It is now well established that two nonallelic eye-color mutants of *Drosophila melanogaster* are deficient in xanthine dehydrogenase. One of these, maroon-like (*ma-l*) is at the extreme right end of the X chromosome, the other, rosy (*ry*), is at locus 51± of the third chromosome. Both mutants exhibit the same eye color (dark red-brown), and are deficient in the enzyme and the enzyme's reaction products, uric acid and isoxanthopterin. The mutants differ in immunological properties, and in a maternal effect, both of which have already been reported (GLASSMAN and MITCHELL, Genetics 44: 153, 547, 1959). This report concerns two otherwise identical *ma-l* alleles (*ma-l* and *ma-l*^{bz}), which can complement one another in *ma-l/ma-l*^{bz} heterozygotes. This complementation results in the production of small amounts of xanthine dehydrogenase, enough of which is present to restore the eye color to normal. However, not enough of the enzyme is present to produce normal amounts of the enzyme's reaction products, or to reduce the accumulation of the enzyme's substrates to levels found in wild type flies. Similar phenomena will be described at the *ry* locus. (This investigation was supported by research grant (RG-6310) from the National Institutes of Health, Public Health Service.)

GOWEN, J. W., J. F. KIDWELL, and JANICE STADLER, Iowa State University, Ames, Iowa: *Third chromosome location of a recessive mutant, pugnose, in the mouse.*—A mutant, designated pugnose (*pn*) arose *de novo* about 1934 in the S stock maintained by DR. J. W. GOWEN when at Rockefeller Institute for Medical Research. The mutant affects specific elements of the skeleton. The frontal and nasal bones are shorter and wider than normal, resulting in the characteristic pugnose appearance. The posterior tip of the sternum is bent ventrally. Pugnose mice are 2 to 4 grams lighter than their normal litter mates. The gene expression is phenotypically distinguishable at 28 days. There is a slightly reduced survival to weaning among the pugnose mice, which is estimated to be about five percent. The males are fully fertile, but there is a marked reduction in fertility of the females. Many do not conceive, and only rarely will they raise a litter, apparently because of a failure to lactate. The mutant behaves as an autosomal recessive. Linkage tests indicate that the gene for pugnose is located in chromosome III, near the spotted gene, *s*. (This work has received assistance from Contract Number AT(11-1) 107 from the Atomic Energy Commission.)

GREGORY, P. W., L. M. JULIAN, and W. S. TYLER, University of California, Davis, California: *Genetic relationships of some bovine achondroplastic mutants.*—Standards for mature weights and heights at hook bones are presented for mature nonachondroplastic control cows (registered Herefords considered of normal size) and for comprest, dolichocephalic, and brachycephalic-achondroplastic mutant stocks, all subnormal in size. Weights and heights are expressed in percentage of control, and each mutant type differs from the control. Even when brachycephalic and dolichocephalic dwarfs do not differ in weight or height, each can be identified by specific head and skeletal characteristics. Reciprocal matings within a breed and between breeds were made between brachycephalic and dolichocephalic mutant stocks, each reported to be conditioned by an autosomal recessive gene. When longheaded dwarf cows were mated to short-headed dwarf bulls all the progeny exhibited reversion to the comprest mutant in weight, height, and general appearance; the reciprocal mating of longheaded dwarf bulls to brachycephalic dwarf cows yielded comprest reversion type progeny and brachycephalic dwarfs in equal proportions. The differences between the matings are statistically significant and indicate that the comprest, brachycephalic, and dolichocephalic mutants are genetically related. A tenable hypothesis is that the three achondroplastic recessive mutants (comprest, brachycephalic and dolichocephalic dwarfs) are differentiated from nonachondroplastic controls by one pair of alleles, *Ss*, and that the distinct classes of achondroplasia are conditioned by the interaction of alleles at two other loci, *a* and *b*, that act as modifiers to gene *s* in the homozygous state. The possible relationship of this achondroplasia to the type studied by CREW (1924) is considered. Some consideration is also given to the biological nature of the alleles implicated.

GRUN, PAUL, and ANNE RADLOW, Pennsylvania State University, University Park, Pa.: *The gene-plasmon interactions of Solanum phureja and S. chacoense.*—F_1 hybrids between these diploid potato species are generally vigorous, normal in development, and fertile. When the hybrids *S. phureja* × *chacoense* are backcrossed by *S. chacoense*, the direction of the backcrossing determines the nature of the progeny. When the hybrid is used as female parent, sterile or morphologically disturbed segregants appear. The nature of the disturbance varies depending upon the *S. chacoense* form used as recurrent parent. The backcross ratios of normal:disturbed are simple ones interpretable as single gene effects. The reciprocal backcrosses, *S. chacoense* × (*phureja* × *chacoense*) are normal in appearance, but varying proportions of the progeny plants are nevertheless sterile, some being male sterile only, some female sterile only, and some both male and female sterile. The male sterility results from degeneration of sporocytes usually at prophase of meiosis. The backcross abnormalities are interpreted as resulting from irregular gene-plasmon interactions. The potentiality of using such backcross data for classifying and mapping of plasmons will be discussed. (Supported by a grant from the National Science Foundation.)

HEED, WILLIAM B., University of Arizona, Tucson, Arizona: *Genetic, cytological and morphological clines in island populations of the cardini species group of Drosophila in the West Indies.*—Ten islands in the West Indies harbor seven closely related species that exhibit a cline in abdominal pigment. The southern island populations own the darkest abdomens. Male genitalia are of four distinct types within the seven species and they also form a cline in relation to the geographic position of the islands.—Unequal sex ratios are characteristic of many interisland crosses. Guadeloupe males × females from islands to the south give progressively fewer males in relation to their distance from Guadeloupe. Barbados females exhibit a maternal effect with males from St. Vincent (60% males, n = 408) and Grenada (88% males, n = 161). Martinique males give a severe Haldane effect with four southern islands (not more than 9% males in any F_1). Martinique females show a maternal effect with St. Vincent (70% males, n = 141) and Grenada (100% males, n = 113).—Five islands in the middle of the Lesser Antilles show one autosomal inversion when crossed to Puerto Rico and St. Thomas to the north, St. Vincent and Grenada to the south, and Jamaica to the west.—Metaphase figures show a clinal readjustment in heterochromatin. The fourth chromosome is a dot in St. Kitts and all islands to the south, a short rod in Puerto Rico and St. Thomas, and a rod equal to length of the X in Jamaica.—The Jamaica population fits both ends of the cline in the Lesser Antilles but in different degrees. It is closest to Puerto Rico in amount of heterochromatin and in structure of male genitalia; it is closest to Grenada in abdominal pattern and genetic similarity (hybrid females × Grenada males is fertile in F_2). (Supported by the National Science Foundation.)

HERSKOWITZ, I. H., Saint Louis University, St. Louis, Mo.: *X-linked recessive lethal mutation rates from Drosophila melanogaster oocytes treated with different dosages and dose-rates of X-rays.*—Three experiments (I, II, III) performed in different months included, besides an unirradiated control (C), four types of X-ray treatment: HQ, 2365r delivered continuously at 215r/min in 11 min; HPA, the same as HQ but with 29-min stops between 11 one-min irradiations, requiring about five hrs for delivery; HPB, 2365r delivered at 23.9r/min in 11 nine-min irradiations alternating with 21-min stops, requiring about five hrs for delivery; LQ, 590r delivered continuously at 215r/min. For C and LQ results from each parental female were kept separately, for other treatments results from pairs of parental females were kept separately. Parents were discarded on the fourth day after irradiation and the standard "Basc" mating procedure was used to detect the mutations.—The mutation percentages obtained were, for I: HQ 3.68 ± 0.41 (2040 tested Xs), HPA 3.47 ± 0.39 (2256), LQ 0.89 ± 0.04 (4519); for II: HQ 4.37 ± 0.45 (2032), HPA 3.54 ± 0.41 (2424), HPB 3.58 ± 0.41 (2011), C 0.11 (1744); for III: HQ 5.67 ± 0.58 (1980), HPB 466 ± 0.29 (4972). Three large, apparently spontaneous, clusters of lethals (one from III HQ and two from III HPB) have been excluded.—These results demonstrate no significant difference between HQ and HP(A or B) or between HPA and HPB treatments *within* an experiment. (Only intraexperiment comparisons are valid.) Statistical analysis of these data indicates HQ could have produced no more than 30% more lethals than HP. It is concluded, then, that these experiments do not support the view that the majority of recessive lethal mutations are produced by multihit events from multiple, independent, tracks of ionization. (Supported by a grant from the U. S. Atomic Energy Commission (Contract AT (11–1)—633).)

HOUSE, VERL L., Ohio State University, Columbus, Ohio: *An analysis of penetrance and expressivity of fourth vein interruption in the Hairless mutant of Drosophila melanogaster relative to variable levels of modifier action.*—Twenty-four generations of brother-sister pair matings with selection for maximal and minimal L_4 interruption of the Hairless (*H*) mutant has produced high and low Hairless lines producing at 26°C 91.7% and 9.2% penetrance respectively in females and penetrance values of 88.3% and 0% in males. An analysis of the relationship between penetrance and expressivity and between penetrance and the degree of bilateral expression over a wide range of penetrance values obtained from parental lines and F_1, F_2 and

backcross populations involving the two lines suggests as valid a threshold interpretation of the variable penetrance and expressivity observed in the data. Making use of this interpretation, the truncated distributions for females of the various populations mentioned above have been transformed into their theoretical normal curves of variation and the means located relative to the threshold for realization of the mutant phenotype. On the transformed scale F_1 and F_2 means are intermediate between parental means, and the means of the backcrosses of F_1 to high and low lines are intermediate between the F_1 and the respective parental means. These data indicate a modification of the relationship of the vein-forming potentiality characteristic of the Hairless substitution to the buffering threshold for phenotypic abnormality of the fourth vein by a polygenic system with additive effects. (Work supported by a grant-in-aid of research of The Development Fund of The Ohio State University.)

HUFF, RONALD L., and JOHN W. GOWEN, Iowa State University, Ames, Iowa: *Effects of partial body X-radiation on learning of inbred strains of mice.*—Male mice of two strains were randomly assigned to treatment groups consisting of control, whole-body, anterior one third, mid one third, and posterior one third X-radiation (400r). The next experiment added four other irradiations, head-mid, head-rear, and mid-rear groups. Mice were adapted in a straight water alley for ten days, then X-irradiated, given a 15 and 22-day recovery period respectively, and then were tested once daily in a modified T-water maze until each mouse reached a criterion of five consecutive correct runs. Trials to criterion, number of errors, and time scores were recorded for each mouse. Regarding trial scores, all radiation groups in one inbred strain (K) differed from irradiated mice in both experiments, but the radiation groups in the other strain (Z) did not differ significantly from their controls. The unirradiated strains differ significantly from each other, Z strain being the superior maze learner. The time scores of irradiated K's showed an increased time per correct trial. The irradiated Z's showed a trend toward increased time per correct trial, but increase was less than that in K strain. Number of errors showed an increase in the K strain. The results show irradiation sensitivity affecting ability of one strain, K, to solve the problem offered them, whereas the other strain was not significantly affected. (This work has received assistance from Contract Number AT (11–1) 107 from the Atomic Energy Commission.)

HUFF, SALLY D., and JOHN W. GOWEN, Iowa State University, Ames, Iowa: *Effect of X-radiation on volitional activity of inbred and hybrid mice.*—Adult male mice of two inbred lines were classified according to level of activity and then given 320r's X-radiation to the following body sections: whole-body, head-third, mid-third, rear-third, and none. Activity levels, as measured by performance in activity wheels, were recorded daily for 24 days following treatment. Mice receiving whole-body irradiation and control mice were retested between the ages of eight and 12 months. Reciprocal hybrids also were tested after treatment with 320r's whole-body X-radiation. Data include responses of only those animals that survived the entire test period. Results indicate that whole-body radiation causes a decrease in activity immediately after treatment. The magnitude and duration of this decrease are dependent on genotype. The two inbred lines can be differentiated from each other and from their hybrids on the basis of normal activity level and response to radiation, an inverse relationship existing between these measurements. Hybrids are more active and show less effect from radiation than either inbred line. The initial test period of 24 days implies that recovery occurs, but retests at later ages indicates the existence of a more permanent effect in one inbred line. Effects of partial body radiation are strain dependent and are not the same for all body thirds, the head-third showing little effect in comparison with the other thirds. No body third is as effective as whole-body radiation. (This work has received assistance from Contract Number AT(11–1) 107 from the Atomic Energy Commission.)

HYDE, BEAL B., University of Oklahoma, Norman, Okla.: *Cytochemical determination of the proportion of protein-bound S-S to -SH in chromatin at three different stages of mitosis.*—The Barrnett-Seligman cytochemical procedure for protein-bound sulfhydryl groups has been employed to determine the ratio of S-S to SH groups in chromatin at various stages of mitosis in the onion root tip. Thioglycolic acid (TGA) was used as a reducing agent; the intensity of stain in reduced and unreduced chromatin and nucleoli was measured photometrically. The cytochemical results indicate that resting chromatin, although variable, has a somewhat higher proportion of protein-bound S-S stain to SH stain (from .21–.31 in three experiments) than metaphase chromosomes (.15–.26). Telophase chromatin, however, appears to have no S-S groups at all since the intensity of reduced and unreduced preparations is not significantly different at this stage. Nucleolar protein has a proportion of S-S to SH groups slightly less than metaphase chromatin (.11–.13).—These results are discussed in relation to what is known about the reliability of this cytochemical procedure. Variability between experiments may depend upon the physiological state of the meristems as well as variations in the cytochemical procedure itself.—The evidence does not support the notion that oxidation of -SH to S-S is involved in the condensation of chromatin during prophase. It is suggested, nonetheless, that the apparent decrease in the S-S/SH proportion from resting stage to metaphase may be caused by a greater resistance of metaphase S-S groups to reduction by TGA. The notion is, however, supported by telophase measurements. Complete reduction of S-S to SH appears to accompany the rapid loosening of chromatin at telophase.

ISHIKAWA, TATSUO (introduced by MARY E. CASE), Yale University, New Haven, Conn.: *Complementation and genetic maps of ad-8 locus in Neurospora crassa.*—A new group of adenine-requiring mutants in *Neurospora crassa*—at the *ad-8* locus—has been located 26 map units to the left of the centromere in linkage groups VI. Two hundred and forty-two primary *ad-8* mutants (obtained from wild type 74A or other closely related wild type strains) and 64 secondary *ad-8* mutants (obtained from the revertant strains at this locus) have been isolated to date. These mutants blocked in the conversion of inosine monophosphate to adenosine monophosphate succinate lack adenylosuccinate synthetase activity. One hundred and six of 242 primary mutants and 19 of 64 secondary mutants gave a positive heterocaryon response with at least one other group of alleles. The resulting complementation patterns indicate the existence of three distinct functional units. Maximal growth of the heterocaryons at 25°C never reaches the wild type rate. In addition all heterocaryons are temperature sensitive at 35°C, exhibiting little or no growth.—Interallelic crosses using 65 primary and secondary mutants at this locus have shown that this locus is composed of at least 19 mutational sites arranged in a linear sequence and the intersite distances are generally additive. Total map distance, based on the prototroph frequency per viable ascospores, is 0.09%. In addition, 12 mutants failed to produce prototroph progeny with two or more linked sites, indicating more than one site was involved in the mutational event. A secondary mutation usually involves damage in the heterocaryon unit in which the primary mutation and the subsequent reversion had been induced. Some of the secondary mutations appear to be located at the same genetic site as the original primary mutation.—A comparison of the complementation and genetic maps of *ad-8* locus reveals a parallelism between the two maps with several exceptions.

IVES, P. T., R. P. HOPKINS, and R. A. CLAY, JR., Amherst College, Amherst, Mass.: *A comprehensive test of the mutagenic effects of combined infrared and γ radiation on successive stages of spermatogenesis in adult D. melanogaster.*—Young males were exposed to infrared at 8°C for 24 hours, then to 1 kr γ. During the following 12 days they were mated daily and singly to successive groups of six Basc or six *vg;se;pol* females. The F_1 were tested for X mutations or Y,2,3,4 translocation combinations. Similar tests were made using males which received no radiation, or only one of the two types, but spent 24 hours at 8°C. Between 200 and 300 tests

were completed for each day (except nearly sterile day 8) in each series and for both X mutations and translocations (T). The γ alone produced the expected pattern of sex-linked mutation frequencies and a similar pattern for T through day 6. The T frequency dropped sharply on day 7. Neither infrared nor 8°C produced a mutagenic effect or influenced γ effects on F_1 sterility and mutagenesis. While X mutations increased 3.5 in days 5–7 over days 1–3 in γ tests. T increased 10× in peak day 6, indicating a higher absolute rate of γ induced mutations in meiosis-spermiogenesis. The X mutation rate was 6 per 10^3 in days 8–12 in γ series while only one T (on day 11) was recovered in 2073 tests in this period. From similar males given 500r γ a higher frequency of X mutations was recovered in days 8–12. This test is being extended to T analysis.

Jaap, R. George, and B. L. Goodman, Ohio Agricultural Experiment Station, Columbus 10, Ohio: *Estimated versus realized genetic correlations in poultry.*—Genetic correlations were calculated from the covariance between and within a large number of full-sister families from a random bred population. Eight-week body weight was observed to be genetically correlated with 16-week body weight (.77), 24-week body weight (.35), 6-months egg production (.15), egg weight at 30 weeks of age (.08), broadness of egg shape (.17) and height of thick albumen in the egg (.12).—A growth selected line was started from this random bred population. After four generations the nine-week weight of females in the growth selected line exceeded that of the random bred by 0.53 lb. These pullets from the growth selected line had a larger body weight at all ages, 0.8 lb. greater than the random bred pullets at 40 weeks of age. Egg production of the selected line was slightly higher during the first month (2.2%) but lower for the next three months of lay (8.6, 5.0, and 4.4%). At 32 weeks of age egg weight was 3 gm heavier, eggs were 2 units broader and albumen was 0.3 mm higher in the growth-selected line. The higher rate of egg production in the growth selected line during the second, third and fourth month of lay was the only estimated response not realized in the selection experiment.

Janick, Jules, and Smritimoy Bose, Purdue University, Lafayette, Ind.: *Heteromorphism involving the "sex chromosomes" of Spinacia oleracea.*—Chromosome I, the longest chromosome in the complement of *S. oleracea* (n = 6), has been shown by trisomic analysis to contain the sex determining factor XY. The "standard" chromosome I is heterobrachial with one arm about twice as long as the other. While this chromosome pair is commonly isomorphic there have been references to heteromorphism in this chromosome pair involving size by Araratjan (1939) and Zoschke (1956), and involving a satellite on the short arm by Dressler (1958). In these cases the nonstandard chromosome was associated with the Y factor.—Cytological investigations have been made on a number of plant introductions of *S. oleracea.* Fourteen out of 36 plants in one line originating in Turkey, designated P.I. 169671, have shown heteromorphism involving chromosome I, one chromosome being considerably longer than the "standard". This longer chromosome I is isobrachial, the added length apparently due to an additional segment on the short arm. This line when induced to flower segregated into 17 pistillate and 19 monoecious plants. There were no staminate plants. Of the 14 plants which were heteromorphic for chromosome I, six were pistillate and eight were monoecious. One monoecious plant heteromorphic for chromosome I has been selfed, and a plant homozygous for the larger chromosome I has been obtained. (This work was supported by The National Science Foundation, G4496.)

Judd, B. H., University of Texas, Austin, Texas: *Further analysis of exceptions arising by recombination within the white region in Drosophila.*—Several combinations of pseudoalleles comprising the white (*w*) series have been shown to yield unexpected recombination types in addition to the usual wild type and double mutant classes. For example, $y^2\ w^a\ spl/w^{Bwx}$ females give $y^2\ w^+$ and $w^{Bwx}\ w^a\ spl$ classes as expected if w^{Bwx} is located left of w^a. In addition a $y^2\ w$ class is recovered. Similar results are obtained from combinations of Brownex with buff, honey

and colored. The unexpected class from the last combination (Brownex/colored) has the same brownish-orange phenotype as the double mutant, but carries the same outside markers as the wild type, and is sterile.—Other pseudoallelic combinations yield only white exceptions such as that reported by GREEN from w^a/w^{a_4}. GREEN interprets the white recombinants as small deficiencies resulting from mispairing and unequal crossing over within the white region. Some of the cases in this study are explainable in this way, while at least one other seems to be more complex. The combination $y^2\ w^a\ spl/w^{bf}$ gives only $y^2\ w$ and $w\ spl$ exceptions. These two white classes are at least partially complementary since $y^2\ w^a\ spl$ recombinants can be recovered from the $y^2\ w/w\ spl$ combination. The white exceptions may be explained by unequal crossing over only if (1) mispairing is more frequent than normal pairing and (2) either both white types represent small but not identical deficiencies or one class is deficient while the other is duplicated with both having the same phenotype. (Work supported by U.S. Public Health Grants C-3648 and RG-6492.)

KADA, TSUNEO, C. O. DOUDNEY, and F. L. HAAS, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas: *Some biochemical factors in X-ray-induced mutation in bacteria.*—Recent studies have established that various postirradiation treatments specifically affecting RNA or protein synthesis are effective in lowering markedly the mutation frequency response to ultraviolet light. Several lines of evidence indicate that DNA synthesis is the terminal event in UV-induced mutation but that phenotypic expression requires protein synthesis subsequent to DNA synthesis.—About half of the X-ray-induced reversions ($tr^- \rightarrow tr^+$) of the tryptophan-requiring auxotroph, *Escherichia coli* strain WP2, are lost when incubated for one hour with chloramphenicol or 6-aza uracil, agents which block RNA or protein synthesis. However, unlike UV-reversion, it appears that a short period of incubation following X-ray exposure before addition of chloramphenicol or 6-aza uracil is necessary for development of the chloramphenicol or 6-aza uracil sensitivity of the mutations. These mutations require RNA and protein synthesis and probably DNA synthesis for induction, in a manner comparable to UV-induced reversion.—The other portion of X-ray induced mutations are not lost when incubated with chloramphenicol or 6-aza uracil for one hour prior to plating on tryptophan-containing medium. These mutations are completely expressed when plated on minimal medium after a short period of incubation in tryptophan-containing medium and before measurable DNA synthesis in the culture occurs. However, expression does not occur in the presence of 6-aza uracil or chloramphenicol. In the case of a thymineless, prolineless double auxotroph, expression ($pr+$) occurs in absence of thymine. The data suggest that, with these mutations, the *functional* reversion is produced by X-ray in the gene without the necessity of DNA replication but that RNA and protein synthesis is required for gene action in phenotypic expression of the reverted character.

KAPLAN, W. D., Department of Genetics, City of Hope Medical Center, Duarte, Calif.: *Autoradiographic and genetic studies of tritiated thymidine in the testes of Drosophila melanogaster.*—During a study on the influence of mating intensity and rate of sperm utilization upon the rate of spermatogenesis in *Drosophila melanogaster*, tritiated thymidine was used in conjunction with autoradiography to label those premeiotic cells which are synthesizing DNA and to follow their development to mature sperm. Additionally, in order to gain some insight into the degree of genetic damage caused by amounts of isotope necessary to produce satisfactory autoradiographs, the frequency of sex-linked recessive mutations was measured.—The autoradiograph patterns obtained indicate that primary spermatocytes reduplicate their chromosomes very early during their life history and move posteriorly in the testes as additional cells are proliferated from the apical spermatogonia. Moreover, the endogenous radiation derived from the incorporated H^3 thymidine exerts a strong mutagenic effect, as determined by techniques for measuring the rate of sex-linked recessive mutations.

KIMBALL, R. F., and STELLA W. PERDUE, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *The relation of the refractory period for mutation induction and nuclear synthesis in Paramecium aurelia, syngen 4.*—X-irradiation of log-phase paramecia during the second half of the interdivision interval produces about one tenth as many recessive lethal mutations as irradiation during the first half. A similar refractory period has been found with α particles and probably with 2537 A ultraviolet. In normal log-phase growth, the beginning of the refractory period coincides with a sudden increase in micronuclear size, presumably chromosome doubling, and with the onset of macronuclear DNA synthesis. The refractory period and macronuclear DNA synthesis continue for most of the rest of the interdivision interval. Growth in the presence of 1.5 mM caffeine delays the increase in micronuclear size and the onset of the refractory period by about two hours but has only a small effect on the time of onset of macronuclear DNA synthesis, though the rate of synthesis is decreased. Conditions in the nucleus after chromosome doubling would not be expected to change the amount of initial radiation damage by a factor of ten; therefore some kind of efficient repair or loss of damage must occur during the refractory period. Previous work has demonstrated metabolic repair of initial damage before the refractory period. Caffeine greatly slows this repair, but the rate increases considerably when macronuclear DNA synthesis starts. The refractory period is probably determined in some way by the doubled state of the micronuclear chromosomes; but the duplicating macronucleus may contribute by increasing the rate of repair, perhaps by furnishing necessary materials or enzymes.

KING, ROBERT L., and ELEANOR H. SLIFER, State University of Iowa, Iowa City, Iowa: *Inheritance of diapause in the eggs of a grasshopper, Melanoplus differentialis.*—Of 100 egg pods laid in the laboratory by *Melanoplus differentialis* (Thomas) females collected near Iowa City 88 contained eggs all of which entered diapause; the remaining 12 pods each contained one or more eggs which hatched without entering diapause. The incidence of eggs with no diapause, in a total of 4,206 viable eggs, was 1.2%. Experiments carried out between 1928 and 1932 had demonstrated that the percentage of eggs which hatch without a diapause can be greatly increased in this species by selection. After seven generations the percentage which hatched with no diapause was raised to 80.1%. The stock, which was still vigorous, was lost accidentally. In 1953 another attempt to isolate a stock with no diapause was begun. In the first generation reared in the laboratory 1.9% of the eggs hatched with no diapause. The percentage rose gradually to 72.5% in the fifth generation and then wavered back and forth between 33.0 and 69.4% during the next seven generations. Viability is low in the stock at present but since this did not occur in the earlier experiment it is believed to be due to the accumulation of genes other than those concerned in the character under selection. It is clear that diapause (or no diapause) is determined genetically in this species. Multiple genes are almost certainly involved and incomplete penetrance or variable expressivity, or both, may account for the difficulty of obtaining a pure-breeding stock.

KIRBY-SMITH, J. S., BENEDETTO NICOLETTI, and MITZI L. GWYN, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *The induction of chromosomal aberrations in Tradescantia pollen by combined X-ray and ultraviolet treatment.*—The modification of X-ray-induced chromatid aberrations in Tradescantia pollen by pre- and posttreatment with monochromatic ultraviolet radiation has been studied. X-ray doses of 50 and 150 rads and ultraviolet exposures at 2650 and 2804 A in the range 0.05 to 0.2×10^6 ergs/cm^2 have been used. The pollen was irradiated under controlled humidity conditions and aberrations examined in the pollen tube mitosis. A pronounced synergism in the action of the different agents has been observed, for both pre- and posttreatments with ultraviolet radiation. The effect is slightly greater for the pretreatment with ultraviolet, but for both pre- and posttreatments at exposures of 0.2 ergs/cm^2, aberration frequencies up to five times the sum of those observed for X-rays and ultraviolet when given alone were found. At the lowest ultraviolet exposure, 0.05×10^6 ergs/cm^2, the synergism is still twice strict additivity, although at this level no significant numbers of aberrations could

be detected in pollen subjected to the ultraviolet radiation alone. The magnitude of the synergistic effect appears to be only slightly dependent on the time interval between the treatments in the period up to four hours. The dependence of these phenomena on LET and ultraviolet wavelength as well as their possible interpretation in terms of ultraviolet induced inactivation of chromosomal rejoining processes will be discussed.

LaChance, Leo E., Brookhaven National Laboratory, Upton, L.I., N.Y.: *The influence of a CO_2 pretreatment on radiation induced chromosome aberrations in Tradescantia.*—Recent studies have indicated that cells irradiated in air plus CO_2 show more chromosome exchanges and interstitial deletions than do those irradiated in air alone. The effect of CO_2 appears only when electromagnetic radiations are used and is completely lacking with fission neutrons. The present study was undertaken to determine if the presence of CO_2 at the time of irradiation is necessary for the enhancement of chromosome aberrations. Inflorescences were pretreated for one hour in air plus CO_2 (equal amounts) and irradiated in air alone. The intervals between the end of the pretreatment and the irradiation were 1, 5, 10, 15, 20, 40 and 60 minutes. Results have shown that the effect of the pretreatment with CO_2 plus air persists after the termination of the pretreatment. The effect decreases linearly during the first ten minutes after termination of the pretreatment, reaches a plateau from 10 to 20 minutes and then drops to control levels in 40 minutes.—Short exposures to carbon dioxide decrease the viscosity of the protoplasm in both plant and animal cells. From previous studies of the CO_2 effect on radiation induced chromosome breaks the hypothesis has been advanced that such a treatment would promote the movement of the breakage ends and favor new reunions over restitution in the original configuration. The present evidence is consistent with this hypothesis. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

Langham, D. G., Genesa, Inc., Maracay, Venezuela: *Breeding sesame (Sesamum indicum L.) varieties to meet specific demands of commercial utilization.*—Close examination of sesame seed pods of numerous varieties demonstrates sufficient genetic variability to make possible the building of sesame lines with different degrees of shattering. Many of these characteristics occur singly, but can be combined by controlled crossing and selection. Thus it is possible to make different combinations of stiff rachis, thin seed pod wall, indented pod wall, rigid internal membrane, long pod, erect pod opening only at tip, seed in blocks, and others, to get the correct degree of shattering corresponding to the potential commercial use of each type of sesame. The pharmaceutical industry, for example, demands sesame oil with an extremely low free fatty acid content which must, therefore, come from sesame seed that has not been scarred in threshing. This requires a shattering pod that permits threshing by simply inverting the bundle. At the other extreme are the large scale farmers who demand an indehiscent variety for mechanical handling. Some of the other industries such as the perfume and confectionery industries, require different degrees of shattering. Various types of sesame resulting from efforts in plant breeding will be available for demonstration.

Law, G. R. J., and W. J. Miller, (Introduced by V. S. Asmundson), University of California, Davis, Calif.: *The A system of turkey blood groups.*—Serological studies of turkeys have revealed 11 factors which can be detected by corresponding isoimmune reagents. Factors named A and H appear to be mutually exclusive; no turkey has possessed both A and H and no turkey has lacked both. Appropriate matings have indicated that H positive birds are homozygous recessive. Red cells of known heterozygotes do not react with anti-H but always react with anti-A. None of the other factors is known to be associated serologically or genetically with A or H. (Research supported by National Science Foundation.)

Leonard, Mary J., George Yerganian, and Henry Gagnon, The Children's Cancer Research Foundation, and Harvard Medical School, Boston, Massachusetts: *Sex chromosomes and "malig-*

nant transformation".—Observations in the Chinese hamster suggest sex chromosome modifications play an important role in "malignant transformation" and transplantation of malignant tumors. L 1290 embryonal male fibroblast derivative is an example of an *in vitro* derivative which displays tumorigenic activity following implantation into cheek pouches of histocompatible hosts. Periodically, sublines were placed *in vivo* as a means of correlating the onset of tumor-forming capacity with the current cytologic observations. After 38 passages *in vitro*, one subline, the Al-90 (aneuploid without new or modified chromosomes) yielded a malignant sarcoma. Each *in vivo* passage was recultured *in vitro*, checked chromosomally, and retransplanted into hamsters. Observations revealed the presence of 6% modified XY chromosomes after the second passage *in vivo*. The percent of modified sex chromosomes rose sharply with each passage *in vivo* and after eight passages (ten months), the sex chromosomes were modified in 100% of the cells. The stemline increased to 25 and there were no indications of structural change among autosomal forms.—Tumorigenic activity (retransplantation) was not fully attained until the frequency of modified sex chromosomes reached 64%. Four clonal derivatives having modified sex chromosomes produced tumors *in vivo*. These continue as successful transplants and return to tissue culture without cytologic alterations. Two other clones, having normal X0 and XY sex mechanisms, respectively, produced tumor-like growths, the first of which regressed after the initial observation and the second failed to transplant *in vivo*. Thus it seems that selection for varied forms of sex chromosomes accompanies tumorigenic expression of tissue previously maintained *in vitro*.

LEWIS, H. W., and H. S. LEWIS, Massachusetts Institute of Technology, Cambridge, Mass.: *Control of dopa oxidase activity by second chromosome factors in Drosophila melanogaster.*—Two distinct genetic mechanisms controlling the quantitative aspects of dopa oxidase activity in Drosophila have been demonstrated. Selection for high and low enzyme activity from a wild type (Samarkand) strain established lines high-2 (790 ± 110 dopa oxidase units) and low-2 (230 ± 50 units), whereas after selection in another strain there was still only the original line, low-3 (70 ± 10 units). Activity in heterozygotes of high-2 $\times$ low-2 approximates the geometric mean of parental activity, suggesting that these two selected lines differ by several interacting modifiers. Activity in heterozygotes of high-2 $\times$ low-3 and low-2 $\times$ low-3 in each case is the same as that of the Samarkand parent. Testcrosses established the presence in low-3 of a locus, alpha-1, which is recessive to its allele in the Samarkand lines. Analysis of crosses of low-3 by a balanced marker strain established that alpha-1 is on the second chromosome and is distinguishable from and recessive to alpha-2 and alpha-4 which are associated with specific balanced second chromosomes.

MCDONALD, D. J., Department of Biology, Dickinson College, Carlisle, Pa.: *The effect of the gene "split" on the fertility of Tribolium confusum.*—Split (*sp*) is a recessive mutation of *T. confusum*. Its most obvious effect in homozygous adults is a severe deformity of the elytra. In addition, it is clear that the fitness of the mutant is far below that of wild type, for in population cages containing both *sp* and its wild type allele, the frequency of *sp* decreased rapidly.—Preliminary observations suggested that both the fertility and longevity of *sp* were depressed. To explore this possibility, over 400 pair matings were made as follows: *sp* ♀ $\times$ + ♂, + ♀ $\times$ *sp* ♂, and + ♀ $\times$ + ♂. Each pair was transferred to a vial of new medium every five days for a total of three transfers. About 63% of the *sp* females were dead at the end of 15 days compared to 6%, 2%, and 0.8% of the *sp* males, + females, and + males, respectively. Where both members of a pair survived 15 days, 59% of the the *sp* ♀ $\times$ + ♂ matings were fertile compared to 96% and 93% of the + ♀ $\times$ + ♂ and + ♀ $\times$ *sp* ♂ matings respectively. Mutant females fertilized during the first five days and surviving through the 15 days still produced only 20% as many offspring as + females.—Further experiments suggest that the impaired fertility of *sp* females is due largely to lowered fecundity and to infrequent copulation perhaps as a result of the *sp* female's deformed elytra. (Supported by National Science Foundation Grant G-8888.)

MAGUIRE, MARJORIE P., University of Texas, Austin, Texas: *Relative positions of chromosomes at pachytene in diploid and hyperploid stocks.*—To explore the question whether synapsis is initiated by the random meeting of moving chromosomes or by a specific force of attraction, a series of studies were made of the relative positions of chromosomes at pachytene, with the exception that these positions might still reflect affinities of the earlier pairing stage. The stocks studied were: (1) maize hyperploid for a Zea-Tripsacum interchange chromosome (the distal half of the short arm of Zea chromosome 2 therefore present in triplicate), (2) maize heterozygous for a Tripsacum segment and also hyperploid as above (the distal half of the short arm of Zea chromosome 2 present in duplicate but one of these located in the extra interchange chromosome), (3) maize homozygous for the Tripsacum segment and also hyperploid as above (the distal half of the short arm of Zea chromosome 2 present only once and located in the extra chromosome), and (4) normal diploid maize. The linear center of each chromosome was used as a convenient, objective index of its location; however, no special properties were attributed to this point. Acetocarmine smear preparations of microsporocytes were studied with the assumption that changes in positions of chromosomes during slide preparation were random and did not affect individual chromosomes selectively. Chromosomes univalent at pachytene were relatively near to bivalents carrying segments homologous to them with frequencies which were statistically significant. The results are not easily reconciled with the hypothesis that synapsis is initiated by random movements. (Work supported in part by the Rockefeller Foundation, National Science Foundation and the National Institutes of Health.)

MANDEL, M., University of Massachusetts, Amherst, Mass.: *Segregation of markers in Escherichia coli-Serratia marcescens hybrids.*—MANDEL, MORTON, DER SARKISIAN and MOHN (Bacteriol. Proc. 1960) have demonstrated that mixtures of auxotrophic mutants of *S. marcescens* HY and *E. coli* 44B yield prototrophic progeny where *E. coli* apparently donates a portion or portions of its genic complement to the recipient *S. marcescens.*—All testcrosses of hybrid progeny with either parent are infertile. The parental phenotype of the recipient is readily reisolated from clones of the hybrids. Hybrid 1 (Hy-1) of phenotype *red, prot, lac*$^+$, *cit*$^-$, *pen*^{s}, *cv*^{s} was derived from the cross *S. marcescens* (*red, met*$^-$, *lac*$^-$, *cit*$^+$, *pen*^{r}, *cv*^{r}) $\times$ *E. coli* (*w, his*$^-$, *arg*$^-$, *lac*$^+$, *cit*$^-$, *pen*^{s}, *cv*^{s}) selected for prototrophy. Hy-1 was infertile in testcrosses with the parental *S. marcescens*, where selections were made for (*prot, cit*$^+$), (*prot, lac*$^+$) or for (*prot, pen*^{r}). Hybrid 2 (Hy-2) of phenotype *red, prot, lac*$^-$, *cit*$^-$, *pen*^{s}, *cv*^{r} was likewise infertile in crosses with the *E. coli* parent. Penicillin resistant cells occur frequently in the cultures of either hybrid, all of which then express the complete phenotype of the parental Serratia. These findings would indicate that integration of the donor genome with the recipient chromosome has not occurred. (This work was supported by a grant from the National Science Foundation.)

MEYER, JAMES R., and NORMAN JUSTUS, Delta Branch Experiment Station and Agricultural Research Service, U.S. Department of Agriculture, Stoneville, Mississippi: *Potential value of doubled haploids in cotton.*—Cotton haploids have been found as small twin seedlings in germinating seeds or as plants in field plantings. These plants with half the usual chromosome number are distinctive. They are miniature plants with small stems, leaves, and flowers. They have no pollen and usually no fruits. Haploids can be multiplied by grafting and their chromosome number doubled with colchicine to produce new doubled haploids. Except for mutation and the possible effects of polyploidy, doubled haploids should be absolutely homozygous "inbred" lines. Variability tests show that doubled haploids are more uniform than ordinary open-pollinated cotton varieties. Agronomic tests show that the best doubled haploids of American Upland cotton (*Gossypium hirsutum* L.) are equal to commercial varieties for lint quality and yield, and that they do not necessarily have narrow ranges of adaptation. At present, doubled haploids in cotton are being increasingly used as genetic constants or "living yardsticks" in many types of laboratory and field experimentation. They are being used as recurrent parents for interspecific gene- and property-transfer work. They promise to aid in the

purification and maintenance of commercial varieties. If F_1 hybrid cottons become a reality, doubled haploids and their derivatives will be of great value as parents.

MILLER, W. J., University of California, Davis, Calif.: *Blood groups in Longhorn cattle.*—Through the courtesy of MR. JULIAN A. HOWARD, Refuge Manager, and DR. D. C. BOSTWICK, U.S.D.A., 99 Longhorn cattle from the Wichita Mountains Wildlife Refuge, Cache, Oklahoma have been blood typed. Genetic diversity and segregation were demonstrated in nine of ten blood group systems. Certain phenogroup frequencies were characteristically different from ordinary cattle in this country. But the most notable difference was in the B system which contained many new phenogroups.

MILLER, W. J., and P. W. GREGORY, University of California, Davis, Calif.: *Coat color, pattern, horn condition and blood groups in cattle.*—With few exceptions, cattle breeds are kept uniform for superficial traits. Therefore, studies for linkage of blood groups with other genetic traits is thwarted. In studies of dwarf cattle, however, it has been necessary to cross breeds and obtain various kinds of segregants.—The sire family of one Angus-Hereford bull has exhibited genetic segregation for black *versus* red coat color, for white face *versus* self pattern, for polled *versus* horned, and for phenogroups in three systems of blood groups. Segregation of the bull's phenotypes was at random with respect to coat color, pattern and horn condition, excluding close linkage with the B, F-V and S blood group systems.

MITTLER, SIDNEY, Armour Research Foundation of Illinois, Institute of Technology, Chicago, Illinois: *The effect of antimetabolites upon the sex ratio in mice.*—Antimetabolites were tested as a possible agent to alter the sex ratio of offspring in mice. Since some antimetabolites block synthesis of nucleic acids, it was thought that an antitumor compound might favor production of a Y-bearing sperm over X-bearing sperm. Amethopterin, a folic acid antagonist, 6-purinethiol hydrate, a purine antagonist, 8-azaguanine, a triazolopyrimidine analog of guanine, and n-methylformamide were injected daily into male mice for several months. These mice were mated prior to and during the period of treatment. There was no significant change in the sex ratio of their offspring. N-methylformamide and amethopterin affected the fertility of some of the male mice.

MOH, C. C., and G. ORBEGOSO, (Introduced by ROBIN L. CUANY), Inter-American Institute of Agricultural Sciences, Turrialba, Costa Rica: *The induction of angustifolia mutants in coffee in R_1 generation by ionizing radiations.*—In studying the effects of ionizing radiations on tropical plant species, it was found that when coffee (*Coffea arabica* L.) seeds were irradiated, a high frequency of angustifolia (*ag*) mutants was induced in the R_1 generation. The *ag* mutants were characterized by producing long and narrow leaves, and the degree of leaf elongation and narrowness varied with different mutants. The *ag* character appearing in the R_1 plants was rarely a sectorial change, but rather the whole plant produced the same characteristic. These mutants have grown for more than a year; none of them have changed back to normal appearance. Thus, the induced changes appear to be permanent.—It has been reported that at least three recessive genes in coffee, when homozygous, give rise to the *ag* character. However, the induction of the *ag* mutants in the R_1 generation can hardly be explained on this basis. Preliminary experiments have shown that the frequency of *ag* mutants increased with radiation dose employed. When thermal neutrons were used as an inducing agent, the *ag* frequency increased linearly with the neutron dose; when X-ray or gamma rays were used, the frequency increased exponentially. This suggests that the *ag* mutants may be due to the result of two-hit chromosomal aberrations. Studies on the effect of fractionated dosage on the *ag* frequency are in progress to test the validity of this hypothesis. (This work was supported by the U.S. Atomic Energy Commission Contract AT(30–1)–2043).

MOORHEAD, P. S., WM. J. MELLMAN, and D. M. BATIPPS, Wistar Institute of Anatomy and Biology, Philadelphia, and Department of Pediatrics, Hospital of the University of Pennsylvania, Philadelphia, Pa.: *Human autosomal anomalies in a sibship of six.*—An improved technique for chromosome preparations employing cells from short-term cultures of leucocytes from human peripheral blood was used in the karyologic study of a family with degrees of developmental retardation and one mongoloid among six siblings.—There is no history of consanguinity between the parents. Both are of normal height, in good health, and neither has a family history of abnormalities of development. Chromosomal analysis reveals the father (age 35) to be "normal", having exact counts of 46 chromosomes and all recognizable pairs and pair-groups identifiable excepting group 6–12 (Nomenclature Conference, Denver, Colorado, 1960). The mother (age 31) has exact counts of 45 chromosomes, possibly resulting from a centric fusion between one medium-sized acrocentric and one small acrocentric. These are tentatively identified as #13 and #22. Similar acrocentrics, also tentatively identified as #13 and #22, were involved in the autosomal translocation reported by TURPIN, LEJEUNE, LAFOURCADE and GAUTIER (Comptes rendus 248: 1, 1959.).—Idiograms indistinguishable from that of the mother were found in each of the three eldest children. The translocation metacentric is approximately the same length as pair #9. The fourth child (female) also reveals counts of 45. However, determination of the particular chromosomes involved is as yet inconclusive. The two youngest siblings show, respectively, a "normal" idiogram and a classic "21-trisomic" mongoloid idiogram with 47 chromosomes.—Preliminary characterizations with respect to speech ability, mental capacity, and other developmental criteria will be discussed in their association with these cytological findings.

MORSE, M. L., University of Colorado Medical Center, Denver, Colorado: *Mapping studies of galactose mutants of Escherichia coli K-12.*—Certain combinations of the galactose negative mutants of *E. coli* in heterogenotes show position effect, i.e., they are mutant in the *trans*-position ($+\,-/-\,+$) and nonmutant in the *cis*-position ($-\,-/+\,+$). During growth recombination occurs in *trans*-position heterogenotes to yield nonmutant *cis*-position heterogenotes. Quantitative study of clones of *trans*-position heterogenotes yields data which can be used to compute "crossover" probabilities between two mutant sites showing position effect. "Crossover" probabilities have been computed for a number of mutant pairs (by the mutation rate estimate methods of LURIA and DELBRUCK) and found to be between $0.047 - 5.8 \times 10^{-4}$ per bacterium per division. Three distinct mutant sites (each of which shows position effect with the others) studied by transducing *from* single mutant donors *to* double mutant recipients in all possible combinations ($a^-b^+c^+ \times a^+b^-c^-$; $a^+b^-c^+ \times a^-b^+c^-$; $a^+b^+c^- \times a^-b^-c^+$) will qualitatively indicate the order of mutant sites, for one combination ($+\,-\,+/-\,+\,-$) will require double crossing over (p about 10^{-8}) to yield nonmutant recombinants ($+\,+\,+$). The double mutants necessary for this three point test are obtained as recombinants issuing from heterogenotes containing two mutant sites. The *Gal*$^-$ mutants of *E. coli* isolated previously and new mutants, spontaneously derived and induced by various mutagens, are being mapped by the two point and three point methods outlined above.

MULLER, H. J., and STANLEY ZIMMERING, Indiana University, Bloomington, Ind.: *A sex-linked lethal without evident effect in Drosophila males but partially dominant in females.*—This "Female-lethal" (symbol *Fl*), of spontaneous origination, has proved to be present in the X chromosome, containing Inversion-49, of all appropriately tested males of a given Indiana University stock, "b33". Females of this stock have attached-X chromosomes. Tests of crossovers between the *Fl*-containing chromosome and other Inversion-49-containing X chromosomes show that *Fl* lies within some three map units of the locus of singed. The only cases previously reported in Drosophila of lethals with unisexual manifestation determined by sex or dosage, rather than (like ordinary sex-linked lethals and like bobbed-lethal) by the simultaneous presence or absence of a normal allele, are the sex-linked female-lethals erect-wings (THOMPSON 1921), at about locus 38, and scute-3 (DUBININ 1928), at zero. Unlike these mutants, *Fl* has in the less

affected sex no, thus far, detected visible or even detrimental effect. In females homozygous for *Fl*, its lethality has always, in counts of several thousand flies, proved to be complete; OSTER reports the homozygotes die as embryos. In heterozygous females, dominance of *Fl* varies from virtually nil to complete, according to invisible features of the genetic background: thus, "b33" males crossed with "b52" females produced 1321 sons and 1215 daughters, but crossed with "b120" females they produced 554 sons and no daughters. Where dominance is high the heterozygous female survivors often show morphological defects or omissions of very diverse kinds. In some crosses the mother's genotype strongly affects the degree of lethality of *Fl* in the heterozygous daughters.—The frequency of gene mutations dominant for lethality requires renewed investigation. (Supported by U.S. Public Health Service Grant RG 2586 [C2].)

MUNDKUR, B., Department of Anatomy, University of Chicago, Chicago, Ill.: *The nature and distribution of submicroscopic basophile particles in yeast.*—Haploid and diploid cells of actively dividing Saccharomyces prepared for electron microscopical study by freezing-drying, disclosed distinct patterns of organization and distribution of basophilia in the nucleus and cytoplasm. The submicroscopic morphological features of these cells were studied in controls employing nucleic acid depolymerases as well as in other controls after postfixation with organic vapors. The results obtained with the techniques employed have revealed vesicular nuclei with no classical chromosomes resolvable at the level of the light microscope. The electron microscope, however, has disclosed a complex organization of submicroscopic nuclear elements whose genetic homologies will be discussed with the aid of lantern slides.

MURRAY, NOREEN E., (Introduced by DAVID D. PERKINS), Stanford University, Stanford, California: *Complementation and recombination studies on me-2 alleles of Neurospora crassa.*—Interallelic complementation has been demonstrated for 12 of 44 methionine-2 mutant strains and the complementation map divides the *me-2* gene into four physiological subunits. The use of marker genes on either side of the *me-2* locus has facilitated the demonstration of interallelic recombination by crossing over and by conversion. A sample of alleles, chosen to represent the different complementation classes, has been linearly ordered on the basis of both interallelic crossing over and total prototroph frequencies. The data are consistent with a linear order of mutable sites within the gene, and suggest that the four physiological subunits of the gene can be translated into linear segments of the genetic map. No correlation was found between the degree of complementation, as judged by growth assays, and the distance between the two mutant sites. Evidence is provided that some alleles have high rates of conversion while others have low rates of conversion.

NAKADA, DAISUKE, (Introduced by DOROTHEA BENNETT), Department of Zoology, Columbia University, N.Y.: *Mutation and the replication of DNA.*—By the use of chloramphenicol and a thymineless mutant of *E. coli*, it was shown that protein synthesis was a prerequisite for the replication of DNA. A residual increase in DNA of 30–50 percent, unaccompanied by protein synthesis, occurs within a few hours of the starvation of an auxotroph for the amino acid it requires. Thereafter the DNA content of the starved population decreases, and after ten days density gradient centrifugation yields no evidence for DNA replication. Nonetheless, during the interval spontaneous mutations take place at a constant rate. The possibility that infrequent exchange reactions occur is under consideration. In contrast, it has been shown that 2-amino-purine will induce mutations in *Salmonella typhimurium* only while DNA is replicating. The role in mutation of DNA replication will be discussed.

NASH, DONALD J., and JOHN W. GOWEN, Iowa State University, Ames, Iowa: *Effects of irradiation while in utero upon postnatal development in the mouse.*—Three inbred strains of mice and their hybrids including reciprocals were used in this study. Pregnant females were exposed to single whole-body X-ray (250 pkv, 30 ma with 0.25 mm Cu and 1 mm Al filtration) dosages from 20 to 320r on 6½, 10½, 14½, or 17½ days gestation as timed by the vaginal plug method.

Response to irradiation was measured by several characters including morphological abnormalities, postnatal growth, lifetime fecundity, and total lifespan, of which body weight response will be reported here. Progeny of irradiated females were weighed at several ages between birth and two and one half months. Body weight response within the over-all population was found to be dependent on both level of irradiation and embryological age at irradiation. Embryological ages in order of increasing sensitivity were 6½, 17½, 14½, and 10½ days. Of treated groups surviving the 75 day period, 320r at 17½ days gestation was most affected, these progeny weighing only 75 percent of controls at 75 days. Growth effects appeared to be permanent since complete recovery was still not evident at 75 days. Genotypic differences in weight response over all treatments reached a maximum around weaning time, but were still present at 75 days. Most of the differences at weaning were due to the early growth advantage of the hybrids over the inbreds. Evaluation of these results emphasize the importance of irradiation *in utero* at all ages on postnatal development. (This work has received assistance from Contract Number AT(11–1) 107 from the Atomic Energy Commission.)

NEWMEYER, DOROTHY, Stanford University, Stanford, California: *Genetic control of enzymes concerned with arginine synthesis in Neurospora.*—Enzymatic studies indicate that the conversion of citrulline to arginine in Neurospora is like that in liver (RATNER *et al.*); citrulline condenses with aspartate to form argininosuccinate, which is converted to arginine by argininosuccinase.—Five mutants at the *arg-1* locus have normal argininosuccinase and little or no condensing activity. This defect appears to be a simple loss, obvious alternatives having been ruled out, and the same enzyme appears to be affected in all cases. When grown under the same conditions (*S*), four mutants at the *arg-10* locus (previously shown to lack argininosuccinase by FINCHAM and BOYLEN) have normal condensing activity.—An apparent exception to the one-gene-one enzyme hypothesis is that, under different growth conditions (*X*), *arg-10* extracts have negligible condensing activity. Here, too, alternatives to simple enzyme deficiency have largely been ruled out. Tests with mixtures of *arg-10 X* and *arg-1* extracts indicate that both lack the same component of the condensing system. Conceivably the inactivity of *arg-10 X* extracts is simply due to the 40% reduction in growth rate that occurs under these conditions; mutants at other loci, grown at this rate, might also yield inactive extracts.—Despite its negligible condensing activity *in vitro*, *arg-10* grown under conditions *X* must be active at some time *in vivo*, since its mycelium accumulates argininosuccinate and no citrulline. In contrast, an *arg-1 arg-10* double mutant, grown under conditions *X*, must be inactive *in vivo* since it accumulates citrulline but not argininosuccinate. The most reasonable conclusion is that only *arg-1* mutants can cause a true block in the condensing reaction. (Supported by grants from the Nutrition Foundation, the Public Health Service, and the American Cancer Society.)

OKADA, TOSHIHIKO, and KEIKO YANAGISAWA, (Introduced by FRANCIS J. RYAN), Department of Zoology, Columbia University, N.Y.: *Elective production of thymineless bacteria.*—When *E. coli* (strain 15 histidineless) are treated with aminopterin, a physiological requirement for thymine is induced. During this treatment the bacteria die, about 10^{-3} surviving after 24 hours at 37°C. When the treated population is provided with thymidine or thymidine and 5-bromodeoxyuridine, growth is reinitiated. The new population can be shown by replication to contain between ten and 50 percent thymineless mutants which prove to be stable. No other auxotrophs are present in similar frequencies. Experiments will be described designed to determine whether, (1) the appearance of the mutants is due to mutation or to selection, (2) other mutants occur with lower frequencies, (3) the mutant factor is located in the *coli* linkage group, and (4) whether the phenomenon is restricted to strain 15 histidineless bacteria.

OLIVE, L. S., and Y. KITANI, (Introduced by J. H. TAYLOR), Columbia University, New York City: *Further studies on aberrant tetrads in Sordaria fimicola.*—A total of seven mutant loci affecting ascospore color have been studied in crosses with wild type to determine whether transreplication occurs at these loci. Asci showing 5:3 and 6:2 segregations for spore color have

been found in crosses involving four of these mutant loci, but not with the other three. It is suggested that the latter group may represent chromosomal aberrations, such as deletions, that are not susceptible to transreplication. With one of the loci (*m*) showing transreplication, all of the abnormal tetrads were of the 6:2 type. A more detailed analysis of the *g* (gray-spored) locus, with a closely linked marker on each side, demonstrates a high frequency of crossing over in the *g* region coincident with transreplication.

O'MARA, JOSEPH G., Department of Genetics, Iowa State University, Ames, Iowa: *The probabilities of complete samples of simple aneuploids.*—The numbers of ways that a sample of simple aneuploids, monosomes or trisomes can occur are given by the coefficients of the terms of $(a+b+c\ldots)^n$, in which the elements of the multinomial represent the individual members of the genome, and n represents the number in the sample. The number of ways that a complete sample could occur is the sum of the coefficients of all terms that contain a complete set of the assigned letters. Since the species in which these chromosomal anomalies are tolerated are generally polyploids, the multinomial may have many elements; since the power to which the multinomial must be raised for the inclusion of many complete samples must be high, the necessary calculations are tedious. One method described by BIZELY for securing the desired value to any desired accuracy can be adapted to this cytogenetic problem. The general term for the number of ways that n aneuploids can include n_a, n_b, n_c, individuals of aneuploids a, b, and c, is $\frac{n!}{(n_a!)(n_b!)(n_c!)\ldots}$. The number of ways in which no member is absent is, therefore, the coefficient of x^n in $n!\ \left(\frac{x^1}{1!}+\frac{x^2}{2!}+\frac{x^3}{3!}+\frac{x^4}{4!}\ldots\right)^g$ in which g is equal to the genome number. This is identical with the coefficient of x^n in $n!\ (e^x-1)^g$. The coefficient of x^n, therefore, is the number of ways that complete samples can occur with specified numbers in the sample. If the number of chromosomes in the genome is low, approximately ten, nine samples in ten will have a complete set if the sample includes four times as many individuals as chromosomes in the genome. If the genome number is high, approximately 20, five times the genome number is necessary for approximately the same result. The probabilities may be secured by dividing these numbers by g^n.

OSTER, I. I., and E. POOLEY, The Institute for Cancer Research, Philadelphia, Penna.: *A comparison of the mutagenic effects of monofunctional and polyfunctional alkylating agents.*—Following our demonstration that representative analogues of the chloroquine and quinacrine mustards containing two chloroethyl groups (i.e., the so-called "two-armed or polyfunctional mustards") are capable of producing genetic changes in spermatozoa of Drosophila, we decided to investigate the mutagenic potentialities of the quinoline derivatives containing only one chloroethyl group (i.e., the so-called "one-armed or monofunctional mustards"). These latter compounds resemble the polyfunctional ones in that they are alkylating agents, have an affinity for nucleic acid, have been shown to possess mitosis- and tumor-inhibiting properties by H. J. CREECH, and are capable of having considerable modifications made in the ring and side chain structures of the quinoline carrier of the nitrogen mustard with a view toward increasing the activity or selectivity of the mustard group. Since in tests with ascites tumors, H. J. CREECH had found a "monofunctional" derivative of quinacrine mustard, 2-methoxy-6-chloro-9-(3-(ethyl-2-chlorethyl)aminopropylamino)acridine dihydrochloride, to be unique in its antitumor activity compared to related analogues, it was decided to investigate the mutagenic effects of this compound. Analysis of spermatozoa derived from adult males (1–2 days old) treated with this "one-armed" mustard yielded 6.0% (44/739) sex-linked lethals in contrast to spermatozoa treated with a similar molar concentration of nitrogen mustard which yielded 1.7% (21/1,224) sex-linked lethals.—Not only do these results add further support to the view that the ability to form chemical cross linkages within or between chromosomes is not essential for the production of genetic changes, but they demonstrate the extent to which the carrier

portion of a nitrogen mustard compound may influence its activity. (This work was supported by a grant from the National Institutes of Health Cy-4615 C1.)

PAPERMASTER, B. W., and S. G. BRADLEY, University of Minnesota Medical School, Minneapolis, Minn.: *Heritability of antibody forming capacity.*—Newly hatched, noninbred Ghostley white leghorns cannot produce antibody immediately following challenge with antigen; however, newly hatched chicks that have received dispersed, hen spleen cells can produce antibody upon presentation of antigen. Each hen spleen was divided into two or more aliquots, stimulated *in vitro* with killed *Brucella abortus* cells as antigen, and transferred to neonates. Serum for antibody measurement was collected six days after transplantation. Some sera agglutinated Brucella at a dilution of 1:5120; other sera, undiluted, had no effect. Sera of chicks receiving cells from the same donor usually had equivalent agglutinin titers. Greater variation in antibody content of sera from chicks receiving splenic cells from different donors was not dependent upon number of viable cells transferred. Indeed antibody titer was largely independent of number of transplanted cells per recipient between 5×10^7 and 5×10^9. Spleens of chimeras, i.e., chicks injected with adult splenic cells and antigen, have been transferred with additional antigen to newly hatched chicks. Generally antibody titers of these (second passage) chicks were less than the (first passage) donors. Rarely however, agglutinin titer of the second passage chimera exceeded titer of its donor. In noninbred animals, host *vs.* graft and graft *vs.* host reactions complicated serial transfer studies. This was partially overcome by splenectomizing recipients or by intravenous administration of Thorotrast.

PETERSON, PETER A., Iowa State University, Ames, Iowa: *Mutable gene control in maize.*—A new mutable gene in maize ($a_1{}^m$) originated in stocks containing pale green mutable (pg^m) (1957 Abstracts). The original pg^m arose in seed exposed during the Bikini A-bomb tests. The significant feature of this new mutable is its relationship to the pg^m system. It has been demonstrated that $a_1{}^m$ is controlled by the inhibitor (I) which causes the A_1 allele to become colorless but, when I is removed by the Enhancer (En), the mutable kernel is characterized by dots of dark anthocyanin pigment on a nonpigmented colorless background. I and En are specific elements of the pg^m system. Thus, it is evident that a previously dominant allele, A_1, has become mutable because of the transfer of specific elements from the Pg locus to this new location, A_1.—In $a_1{}^m$, a wide array of very distinct patterns (due to the timing and frequency of mutation events, i.e., late and early, high and low frequency and combinations of these) have been isolated. From these individually identifiable patterns other distinct patterns arise. Tests show that control of the pattern is intrinsic to the mutable allele itself. It is a study of these patterns, their derivatives and the sequence of changes which occur that should provide significant information about the nature of mutable gene elements.—As described above, two elements are necessary for mutability (as in $a_1{}^m$) – I, the inhibitor of the locus and, En, the Enhancer that in turn controls removal of the inhibitor. Pattern changes are believed to result from alterations in the basic composition of the inhibiting element, I. This may tentatively be called the two-factor hypothesis. Perhaps it is possible, however, to explain mutable gene activity in another way. This may be called the one-factor or positional hypothesis. Instead of two factors being necessary, a position change involving just one element could conceivably control pattern changes (i.e., the position of Mp in BRINK's P^{vv} series).—Experimental data and additional results will be discussed.

PHILLIPS, J. N., JR., USAF Aerospace Medical Center (ATC), Brooks AFB, Texas: *Modification of mutation frequency in microorganisms through use of multiple mutagen treatment.*—Recent advances toward resolution of the mechanism of establishment and fixation of mutations in selected strains of *E. coli* has led to statement of a general hypothesis that the time of establishment of an induced mutational event is prior to synthesis of the affected self-replicating material. To test the applicability of this hypothesis for other types of microorganisms, procedures have been derived through which three separate mutagens may be applied separately, in pairs,

or all together in a single procedure. Two diverse microorganism types, actinomycetes and algae, have been studied. Background or spontaneous mutation frequencies have been compared with observed frequencies when spore or cell populations are treated with ultraviolet light, RNA and DNA analogs, and/or alkylating agents. It has been found that in some populations large increases in mutation frequency may be achieved if mutagen treatment(s) is properly integrated with cell division events. Timing of procedural events is extremely critical and can drastically alter results. Critical procedural factors include premutagen treatment, cell synchronization, mutagen treatment, and posttreatment management. The mutagens tested are approximately additive when utilized in pairs, but appear to act synergistically when all three are utilized within any single procedure. Tentative conclusions and delineation of work to be undertaken are discussed.

PIERRO, LOUIS J., The California Institute of Technology, Pasadena, Calif.: *Strain differences in tyrosinase in the eye of the mouse.*—The tyrosinase content of the eyes of unrelated piebald and C57 Black mice were compared by assaying aquaeous extracts of homogenized eyes for tyrosinase activity. In extracts prepared from eyes of varying stages from birth through the first postnatal month, greater activity per eye was always obtained from C57 Black mice. Differences were most marked during the 8–20-day period.—Determination of the protein content of extracts demonstrates that the extractable protein increases linearly during the 4–20-day period and that similar values are obtained for corresponding stages in both strains. Calculation of specific activities (activity per mg protein N) gives evidence of a general decline during the postnatal stages. The pattern is interrupted in the C57 Black extracts by a significant increase in specific activity of preparations from eyes of animals 12 days of age. After this time specific activity actually decreases more rapidly in the C57 Black preparations than in those from the piebald strain.—The increase in specific activity in 12–day extracts is interpreted as an exceptional ability of the eye of the C57 Black mouse for tyrosinase synthesis during the 8–12-day period. It is suggested that the potential for a considerable increase in tyrosinase content between 8–12 days is a more significant difference between the two strains than is the apparent greater tyrosinase content of the eye of the C57 Black mouse. (Public Health Service Research Fellow of the National Cancer Institute. Present address: Biology Department, Wheeling College, Wheeling, West Virginia.)

PIPKIN, SARAH BEDICHEK, The Gorgas Memorial Laboratory, Panama City, Rep. of Panama: *Sex combs in unbalanced male forms of D. melanogaster.*—A study was made to determine if the sex combs of hyperintersexes bearing 2X3A + a fragment of chromosome 3 showed developmental modification in the male direction. Mean numbers of sex comb teeth of hyperintersexes compared with their sibling control 2X3A intersexes were as follows: 12 L hyperintersex, 6.50 ± .17; control sib intersex, 7.58 ± .16; 85C L hyperintersex, 6.09 ± .16; control, 6.99 ± .14; 2 R hyperintersex, 7.50 ± .24; control, 7.40 ± .15; 30 R hyperintersex, 8.00 ± .23; control, 7.58 ± .22; 6 R hyperintersex, 8.12 ± .23; control, 8.00. Thus the mean number of teeth per sex comb in hyperintersexes is either very close to or slightly less than the mean of the 2X3A sib control intersexes. Hence, no shift toward maleness is found in hyperintersexes according to the development of their sex combs.—The mean sex comb tooth number of hypodiploid males of the genotype 1X2A—the section between T(3,4)H7 and T(3,4)A13 was 8.57 ± .14, control diploid males, 11.0 ± .15. Hyperdiploid males (1X2A + fragment of 3), representing chromosomally unbalanced forms where no sex shift is expected, were studied for comparison with the other aneuploids. Hyperdiploid males carrying 2 R in excess of 1X2A showed a mean sex comb tooth number of 9.27 ± .11; control diploid males, 11.0 ± .15. Three H7 L hyperdiploid males had combs with a range of from 6–9 teeth. A single 12 L hyperdiploid male had seven teeth in each comb. Super males (1X3A) showed a mean sex comb tooth number of 8.56 ± .22. Thus a reduction of the number of sex comb teeth is generally found in chromosomally unbalanced male forms. This reduction is owing to a disturbance of growth processes due to genic imbalance.

RAI, K. S., University of Chicago, Chicago, Ill.: *Localization of genes in Collinsia heterophylla by the trisomic method.*—Aneuploids of *Collinsia heterophylla* Buist ($n = 7$) were found in the progeny of 13 plants treated with colchicine. Diploid, recessive plants were crossed with tetrasomes ($2n + 2$) carrying dominant alleles as male parent. Simplex (AAa) trisomics and hybrid disomics were either self-pollinated or crossed with recessive diploids. Tubular leaf (*lt*) showed distorted ratios with aneuploid lines F, J, and L, white flower-1 (*w-1*) with line B, and white flower-2 (*w-2*) with lines E, M, and J. Furthermore, *lt* yielded normal ratios with lines A, B, D, and K, *w-1* with A, C, E, H, and K, and *w-2* with D, F, and K. Data from crosses involving these genes and certain aneuploid lines did not fit either category. Line J gave unexpected results in crosses involving *lt* and *w-2*. In some crosses, disomic hybrids did not yield the expected ratio when self-pollinated. The trisomic method for localizing genes in *C. heterophylla* does not seem to conform with results obtained in other species. The transmission of extra chromosomes in this species also does not conform with observations on extra chromosomes in other species. (Aided by grants from the National Science Foundation and the Abbott Memorial Fund, The University of Chicago.)

RAMEY, HARMON H., JR., Delta Branch Experiment Station, Stoneville, Mississippi: *Evidence for gene interactions in the inheritance of lint length in upland cotton (Gossypium hirsutum L.).*—The F_1 mean for a character is expected to equal the average of the two parents when the gene action is additive. Gene interactions would cause the F_1 mean to deviate from the midparent. The F_2 mean would fall halfway between the F_1 mean and the midparent when allelic gene interactions are involved. When the F_2 mean deviates from this expected position, nonallelic gene interaction is indicated.—Lint length data were obtained from the parental, F_1 and F_2 generations of a cross of Half and Half and Delfos 9252. The average lint lengths, in inches, were: P_1 (Half and Half), 0.88; P_2 (Delfos 9252), 1.08; F_1, 1.19, F_2, 1.18. Biometrical analysis of the data indicated that the deviation of the F_1 mean from the midparent and the deviation of the F_2 mean from the mean of the F_1 and midparent were not due to chance only. This suggested the possibility of both allelic and nonallelic gene interactions being involved in the inheritance of lint length in this population of Upland cotton.

RÉDEI, GYÖRGY, University of Missouri, Columbia, Missouri: *Genetic control of 2,5-dimethyl-4-aminopyrimidine requirement in Arabidopsis thaliana.*—This autogamous ($2n = 10$) Crucifer is at present the only angiosperm amenable to studies of gene action on the metabolic level. Subvital mutants can be raised to maturity on chemically defined media in aseptic test tubes with 8–10 generations per year.—A recessive hereditary change, induced by X-rays, involves seedling lethality unless 2,5-dimethyl-4-aminopyrimidine (*Py*) is supplied. Thiamin and its phosphoric esters and acetylthiamin also satisfy the nutritional requirement. The antimetabolite neopyrithiamin is also utilized because it has the proper *Py* moiety. The antivitamin oxythiamin is inactive due to the OH substitution in position 4 on the pyrimidine ring. The thiazole moiety of thiamin has no effect. If an appropriate amount of *Py* is present in the medium, the recovery of the mutant is complete; fertility is normal in both male and female. The sensitivity of the mutant toward *Py* is great: 0.001 microgram thiamin provokes a response. If the requirement is not completely satisfied, a particular and clear-cut pigmentation deficiency is found, permitting identification in almost any genotype. The pattern of inheritance has been somewhat anomalous. In the X_2 there were only 16 plants, of which nine were wild and seven lethal. In the X_3 there was a variation among families: some segregated 3:1, others behaved like the X_2, and in some others the mutant class slightly but definitely outnumbered that of the wild. Present evidence does not point to the presence of a lethal gametophyte factor on the "wild" homologous chromosome impairing its transmission. Mutation, at an unexpected high frequency, could be detected in the families segregating for the *Py* requirement. (Contribution from the Mo. Agri. Experiment Station, Journal Series No. 2150. Approved by the Director.)

RILEY, H. P., and DEBDAS MUKERJEE, University of Kentucky, Lexington, Ky.: *Chromosomes in Agapanthus.*—As part of a cytotaxonomic study of Agapanthus, a genus of Amaryllidaceae endemic in South Africa, chromosomes were counted and measured from five species and two unidentified plants. They were obtained from root tips of seeds or bulbs of wild plants shipped directly from that country. Chromosomes were measured and classified into the following groups: a = 9–11.5 micra; b = 8–8.9; c = 6–7.9; d = 5–5.9; e = 3–4.9; B = supernumerary chromosomes. The diploid numbers and the measurements of the chromosomes of the various species are: *A. campanulata*, 6c + 8d + 16e = 30; *A. minimus*, 6a + 2b + 8c + 8d + 6e = 30; *A. orientalis*, 2a + 4b + 8c + 8d + 10e + 2B = 32 plus two supernumeraries; *A. inapertus*, 10a + 2b + 12c + 6d = 30; *A. praecox* 6a + 2b + 10c + 8d + 6e = 32; *A.* sp. 148/55, 2a + 10c + 4d + 14e + 2B = 30 plus two supernumeraries; *A.* sp. 71/50, 6a + 5b + 8c + 8d + 2e = 29. In *A. orientalis*, the two supernumeraries are prominent in the emerging radical but were not observed in root tips that were several days old or in root tips from bulbs; but in *A.* sp. 148/55, they were present in all stages. (This work was supported by Grant G-5677 of the National Science Foundation.)

ROSS, J. G., M. E. SANDERS, and C. J. FRANZKE, South Dakota State College, Brookings, South Dakota: *Asynapsis in sorghum.*—A mutation for asynapsis, *as*^{2}, inherited as a single Mendelian recessive, occurred as a chimeral sector in a sorghum plant of variety Experimental 3. Large regions of pachytene chromosomes were unpaired, and evidence indicated that complete pairing had not occurred. Univalents, which varied in number from 0–12, were found to lag at anaphase I and to be subsequently pulled apart by neocentric action. Transverse or longitudinal separation at the centromere resulted, depending on the orientation of the univalent at the equator. Univalents in which chromatids divided at first division were in certain instances torn apart at anaphase II to form fragments. If cytologically marked chromosomes were obtained from asynaptic material, they would be valuable for testing the theory of somatic reduction after colchicine treatment. The gene for asynapsis may also be valuable as a tool in studying the mechanism of synapsis.

RUDNER, RIVKA, (Introduced by L. C. DUNN), Department of Zoology, Columbus University, N.Y.: *Mutation as an error in base pairing.*—Base analogues and nitrous acid were used as mutagens in studying the trytophane-D locus of *Salmonella typhimurium* where 11 sites have mutated to a condition causing auxotrophy. These sites reacted differently, some being unresponsive while others responded to one or more mutagens and to different extents. Results such as these have been interpreted as mistakes in base pairing during the reproduction of DNA. To test this hypothesis the bacteria were synchronized by fractional filtration. Determinations were made of the numbers of viable parents and mutants and of the DNA per culture. It was found that the mutants appeared in a pattern determined by DNA replication and consistent with the hypothesis that the analogue in question, 2-aminopurine, was incorporated during replication and rarely chose an erroneous base upon subsequent replication. This correspondence with theory was found whether the 2-aminopurine was present continuously or only during the first replication, or whether the cells were allowed to replicate their DNA without cell division. A number of other hypotheses were entertained, among them the notions that the mutation is completed in one step, that the pairing error is made at the time of incorporation, and that some normal base instead of 2-aminopurine is initially incorporated. None of these hypotheses fit the facts. Chemical investigations on the incorporation of 2-aminopurine into DNA are under way.

RUSSELL, LIANE BRAUCH, and JEAN W. BANGHAM, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *Further analysis of variegated-type position effects from X-autosome translocations in the mouse.*—In an earlier report of a case of variegated-type position effect at the *b* locus (RUSSELL and BANGHAM, Genetics 44: 532, 1959), it had been inferred from preliminary indications that the chromosomal change involved was a translocation between chromosome

8 and the X chromosome. Linkage tests now completed with the sex-linked marker *Ta* (on *b/b* background) support this earlier conclusion. Frequency of crossing over between the breakpoint and *Ta* is about 8%.—In maintenance of the brown-variegated stock, semisterile black (i.e., nonvariegated) females, an unexpected type, occasionally appear. These exceptional animals, in tests with *Ta* males, have yielded hemizygous *Ta* daughters, indicating that they are deficient for a portion of the X chromosome containing the *Ta* locus, or may even lack the entire X chromosome. It can thus be inferred that absence of one X chromosome, or X-chromosome portion, suppresses variegation of $+^b$. This would also explain why male translocation heterozygotes are black, rather than brown-variegated (as are translocation females bearing two complete X chromosomes).—In a second case of position effect involving variegation at the *c* locus, results from matings of variegated females with c^{ch}/c^{ch} males are completely analogous to those described earlier for *b* locus variegation. It is, therefore, concluded that the chromosomal basis is a translocation between the X and chromosome 1. The two cases differ in proximity of the break to the locus involved: crossover frequency for the *b* and *c* variegations are 16% and $< 1.5\%$, respectively.

SAND, SEAWARD A., Connecticut Agricultural Experiment Station, New Haven, Conn.: *Relation between Sachs' grand period of growth and mutation at two loci in Nicotiana.*—Clone S5230–5, selected from cultures of interspecific hybrid origin, was employed here as used previously in studies of temperature and chronic gamma irradiation effects on mutation. This clone is heterozygous for the mutable *V* gene (v_{83}/v_s) and for the stable *R* gene (*R/r*), which affect flower color. Replicated cuttings of the clone were grown in sand culture under conditions of controlled temperature and light. The somatic effect on a mutable gene system was compared with a stable gene system in the same tissue by counting speckled sectors (changes from v_{83} to v_s phenotype) and purple sectors (changes from *R* to *r* phenotype) on flowers from successive nodes of the main flowering axis. Similarly, germinal effects on the same gene systems were studied by counting speckled and purple individuals in progeny obtained by self-pollinating flowers at selected node positions.—Somatic sectoring rates for both the mutable and stable genes show direct correlation with the natural change in rate of growth of the flowering branch. Sector frequencies at mid-branch are about twice as high as for proximal and distal nodes.—We have scored 1,200 progeny in 14 families distributed from 6 to 96% of the total nodal range. These preliminary results suggest a node position effect on frequency of speckled individuals of about 10%, with the *minimum* observed in families from mid-branch pollinations, which, however, give the *maximum* purple progeny frequencies.—Effect of metabolic environment on a measured change in somatic expression of two genes is indicated, and in addition, the progeny results suggest effects on mutation of these genes.

SARVELLA, P. A., and R. A. NILAN, Mississippi State University, State College, Mississippi, and Washington State University, Pullman, Washington: *The relation of the origin and number of tillers of X_1 plants to induce mutation frequencies in barley.*—In radiobiological studies using barley, induced mutation frequencies are measured by the number of chlorophyll-deficient X_2 seedlings observed one generation following irradiation of the seed. Thus, changes in the growth and development of the X_1 plant, which is a mutation chimera, can alter the detectable mutation frequencies. Of particular interest has been the influence of plant tillering on mutation frequencies (H. GAUL, Flora 147:207–241, 1959) which has led to the diplontic selection theory.—The present study was designed to determine the relation of the origin and number of tillers to detectable mutation frequencies in Haisa II barley (2-rowed). The viability of buds and subsequent tillering in the irradiated seed were studied with the aid of tetrazolium chloride, a vital stain. Plant survival and emergence were found to be benefited by low amounts of radiation and decreased with higher. Mutation frequencies in the X_2 seedlings were calculated on a plant, spike, and seedling basis depending on the origin of the X_1 tillers and tiller groups. Each method of measuring showed that irradiated plants with more tillers also contained more mutations

except when only the apical meristems were scored. This is contrary to the above theory of diplontic selection. Possible explanations for this discrepancy will be discussed.—The method which most closely approached linearity was on a per spike basis. Deviations from linearity seem to be related to a decrease of tillering and the origin of the tiller groups at irradiation.

SCHAEFFER, G. W., F. A. HASKINS, and H. J. GORZ, University of Nebraska, Lincoln, Nebraska, and Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture: *Genetic control of β-glucosidase activity in Melilotus alba.*—Four essentially isogenic lines of *Melilotus alba* were used in this study. With respect to *Cu/cu* and *B/b*, the two major gene pairs governing level and form of coumarin, the four lines had the following genotypes: *CuCuBB, CuCubb, cucuBB, and cucubb. CuCuBB* plants contain both free and bound coumarin (apparently a glucoside of *o*-hydroxycinnamic acid); *CuCubb* plants are high in coumarin, but essentially all the coumarin is in the bound form; and *cucuBB* and *cucubb* plants are low in both forms of coumarin. Crude homogenates of various plant tissues, supernatants from high-speed centrifugation of crude leaf homogenates, and acetone precipitates from the foregoing supernatants were used as enzyme preparations for β-glucosidase assays. As substrates, *p*-nitrophenyl-β-D-glucoside and chromatographically purified bound coumarin were used. The K_m value for *p*-nitrophenyl-β-D-glucoside was approximately ten times as high as that for bound coumarin. Preparations of leaf tissue from *BB* plants were high in β-glucosidase activity, while in comparable preparations from *bb* plants the activity was negligible. In preparations of various tissues from *BB* plants level of β-glucosidase activity was closely associated with content of free coumarin. The hypothesis is proposed that in the Melilotus plant free coumarin is formed through the action of β-glucosidase on bound coumarin and that the *b* allele influences coumarin metabolism through its effect on the enzyme, β-glucosidase.

SCHAIBLE, ROBERT H., and JOHN W. GOWEN, Iowa State University, Ames, Iowa: *Delimitation of coat pigment areas in mosaic and piebald mice.*—In our laboratory 6%, 10%, and 96% of the heterozygotes of Mi^{wh}, W^a, and *Va* respectively are mosaics exhibiting wild type areas on an otherwise diluted coat. The large altered regions conform to a regular pattern of six bilateral (nasal, temporal, aural, costal, lumbar, sacral) and two medial (coronal, caudal) pigment areas. —Each wild type area is assumed to be pigmented by melanocytes proliferated from a single primordial pigment cell having an altered genotype. Shape of the spots also indicates that melanocytes have spread out from the centers of the areas during final stages of migration from the neural crest. In *ss* $Mi^{wh}/+$ and *btbt* $Mi^{wh}/+$ stocks, mosaic individuals, which display either a wild type or a dilute spot on a white background within each pigment area, are evidence that both types of spots develop in like manner. Low frequencies of mosaics in these compound genotypes (1% and 3%, respectively) indicate that restriction in the size of the pigmented spots has reduced the number of melanocytes which can be altered genetically.—Irradiation *in utero* (150r X-ray at ten days gestation) raised the frequency of mosaic individuals (*bt*/+ $Mi^{wh}/+$) from 9% to 25%. The effect of *bt* and *s* loci on the frequency of $Mi^{wh}/+$ mosaics demonstrates the importance of considering genetic background when estimating natural or radiation induced "somatic mutations". (This work has received assistance from Contract Number AT[11–1] 107 from the Atomic Energy Commission.)

SEECOF, R. L., The University of Texas, Austin, Texas: *The effects of ploidy changes on enzyme activities in Drosophila melanogaster.*—Spectrophotometric assays were developed which allow several enzyme activities from Drosophila homogenates to be measured quantitatively. Guanase, alcohol dehydrogenase, alkaline phosphatase and fumarase activities were measured (on a protein basis) and found not to differ among males, females and superfemales. The level of aconitase activity was found to be highest in females, lower in males and lowest in superfemales. The five enzyme activity levels in males lacking a Y chromosome were not different from those in normal males. No differences have been detected between the *melanogaster* strains

examined so far. The results to date suggest a factor which is present on the X chromosome, but missing from the Y, that influences aconitase activity. However, the change in activity in superfemales is opposite to that expected if it were due to a simple additive effect from X chromosomes. Experiments are now being performed to determine the activities in triploid females, in flies with extra Y chromosomes and in flies with selected duplications or point mutations.

SHREFFLER, D. C., (Introduced by R. D. OWEN), California Institute of Technology, Pasadena, Calif.: *Genetically determined plasma protein variants in the mouse.*—Sera from 12 inbred lines have been compared by starch gel electrophoresis. The CBA line differs from the other lines in one major β-globulin component, β^1, which migrates more rapidly than β^2, present in the other lines. Serum from F_1's of reciprocal crosses between CBA and C57BL (which has β^2) contains both proteins in approximately equal, but reduced, quantities. F_2 and reciprocal backcross progeny yield the two parental and the F_1 phenotypes in the frequencies expected with genetic control by a pair of codominant alleles. The trait is not detectably linked to the agouti or hemoglobin loci. Tests for linkage to other markers are being made. Electrophoretic differences in certain minor β- and α-globulin components have also been observed among the inbred lines tested and within a stock of noninbred mice, however these differences have not yet been entirely resolved.—Sera from nine lines have been tested against a rabbit antimouse globulin serum by diffusion in Ouchterlony plates. With this reagent, a difference has been detected between three lines, CBA, C3H and AKR, and the remaining six, in the position of the precipitin band for one component. The position of this band for the three variant lines is equivalent to that obtained with a 1/8 dilution of the serum of the other lines. In CBA $\times$ C57BL F_1's, the position of the band is intermediate under appropriate test conditions. Preliminary results in F_2's and backcrosses indicate that this effect is inherited as a single gene difference, unrelated to the β^1–β^2 electrophoretic difference between these two lines.

SKAAR, P. D., Montana State College, Bozeman, Mont.: *Host genes determining a reciprocal phage modification system.*—A large fraction of P1b coliphage particles grown on *E. coli* B/r are incapable of forming plaques on *E. coli* K-12, although all adsorb. The distribution of K-12-competent phage in individual bursts on B/r indicates that, if they are genetic variants, their frequency of origin is extremely high. Within one cycle of growth on K-12, a P1b population is obtained with reciprocal properties. The role of the host is difficult to assess in this system. Either the host merely screens for a frequent phage mutant after adsorption, or it actively influences the phenotype of genetically uniform phage which parasitize it. The latter interpretation, though less simple, is favored by its validity in similar, but nonreciprocal, systems. Crosses of K-12 and B/r strains generally yield recombinants which resemble B/r in their relationship to P1b. However, where selection screens for recombinants carrying the *L* (leucine) allele of the K-12 parent, a high proportion of the recombinants are like K-12 in their relationship to P1b. Whatever the host's action, the genetic basis of this action is highly localized in the bacterial genome. (Supported by a grant from the National Cancer Institute.)

SKAAR, P. D., and N. E. MELECHEN, Department of Zoology and Entomology, Montana State College, Bozeman, Mont., and Department of Microbiology, St. Louis University School of Medicine, St. Louis, Mo.: *Hypertrophy of phage production following thymine starvation.*—Thymineless *Escherichia coli*, B-3, infected with bacteriophage P1b fail to complete the lytic cycle in the absence of thymine. A small amount of long-delayed lysis is attributable to "leakiness" of the bacterial block. Yet under thymine-free conditions, processes readily occur in the infected cells which facilitate phage formation when thymine is later added. Specifically, if phage are mixed with bacteria in thymine-free medium at time 0, a burst of 5–10 phage per infected bacterium occurs at 75 minutes if thymine is added at any time between 0 and 45 minutes. If thymine is added after 45 minutes, a burst occurs 30 minutes after the time of this addition and the burst size is directly proportional to the time of preincubation (minus 35 minutes) in thy-

mine-free medium. A maximum burst size of 50–70 is observed after 180 minutes preincubation. —The data can be described in terms of two steps in phage production: (1) A thymine independent step which results in the accumulation of some substance influencing eventual burst size, and (2) a thymine dependent step which takes at least 30 minutes.—Isotopic studies show that (1) DNA synthesis is associated with the second and not the first step, and (2) a substance is formed during the thymineless preincubation which shortens the interval between the addition of thymine and onset of DNA synthesis. (Supported by grants from the National Cancer Institute and the National Foundation, respectively.)

SOKOLOFF, ALEXANDER, EARL L. LASLEY, and ROBERT R. SHRODE, William H. Miner Agricultural Research Institute, Chazy, New York: *A map of the X chromosome in Tribolium castaneum Herbst.*—Among a number of new mutants appearing spontaneously in the flour beetle, five have been found to be linked in the X chromosome: red (*r*), a recessive mutation, replaces the normally black color of the eye; miniature appendaged (*ma*), a semilethal mutation, reduces the elytra and membranous wings to about two thirds of the normal length, and the podomeres of all the legs become short and thick; pygmy (*py*) is an incompletely recessive gene reducing the length to two thirds that of the normal, or the weight to about half the normal weight; spotted (*sp*) is an incompletely recessive gene overlapping wild type in its expression and best detected in young (not fully colored) imagoes—it prevents the deposition of the chestnut color over variable portions of the elytra; truncated elytra (*te*) is a lethal gene causing a variable folding of the elytra. Experiments are under way to localize the last gene. Taking paddle (*pd*), a previously described sex-linked gene, as the starting point, the relative position of the other genes is:

sp —— *py* — *ma* —— *r* — *pd*

The distance between *pd* and *r* is about one unit, between *ma* and *pd* about 12 units, between *ma* and *py* about two units, and between *pd* and *sp* about 50 units.

STADLER, JANICE, and JOHN W. GOWEN, Iowa State College, Ames, Iowa: *Inheritance as a factor in life spans of mice when exposed to different irradiations.*—Ten strains of inbred mice have been irradiated at 46 days of age with dosages ranging from zero to 960r 98 pkv, 2.5 ma no filter and to 640r 250 pkv, 30 ma filter 0.25 mm Cu + 1 mm Al radiations. The mean life spans of 0r mice ranged between 317 days to 662 days for the different strains. Males had somewhat longer life spans than females. Longer wave length X-rays were less effective in reducing life span than 250 pkv. Passing over the intermediate radiations, the 640r 98 pkv and 320r 250 pkv caused clearly distinguishable reductions in average longevity. 960r and 480r for their respective kilovoltages caused the more susceptible strains to die soon after irradiation. The strain longevities ranged from 293 days to 550 days and 55 days to 300 days, respectively for the different radiation dosages. Males and females had closely similar longevities at intermediate dosages with the females having longer lives at the highest dosages. Strain differences are noticeable but evidently not for the same reasons. Thus one strain has a long life span when untreated and comparatively long when treated; another is long-lived untreated but very short when irradiated with high X-ray dosages. Another strain has medium life untreated and is relatively long-lived when treated. Intrinsic factors affecting duration of life are numerous; some have been worked out. (This work has received assistance from Contract Number AT(11–1) 107 from the Atomic Energy Commission.)

STALKER, H. D., Washington University, St. Louis, Mo.: *Relationship of meiotic drive and inversion association in Drosophila paramelanica.*—In wild populations of *D. paramelanica* the four X-Left·X-Right inversions: AB·CB show non random association; nearly all chromosomes being ++·++ (Standard sequence) or AB·CB, with the rare recombinant types produced by crossing over apparently eliminated as they are produced. AB·CB chromosomes are associated with the sex-ratio trait (AB·CB/Y males may produce nearly all X-bearing sperm and thus

female progeny). Two kinds of sex-ratio chromosomes exist; Southern Type, in which the trait is suppressible only by autosomal modifiers and not by Y chromosomes, and Northern Type, suppressible by autosomal modifiers and Southern Type Y chromosomes. (Northern Type Y chromosomes do not suppress sex-ratio). Chromosomes of karyotype ++·CB and ++·+B derived by crossing over from Southern Type AB·CB retain sex-ratio characteristics, but change to Northern Type, suppressible by Southern Type Y which is characteristic of populations in which such recombinants would normally appear. AB·++ and AB·C+ show no sex-ratio characteristics. None of the recombinant types: AB·++, +B·++, A+·++ and ++·CB produced by crossing over from Northern Type chromosomes showed any sex-ratio characteristics. Apparently the breakdown of Northern Type AB·CB results in loss of the trait. Thus dissociation of either type of AB·CB complex results in suppression of sex-ratio and loss of meiotic drive characteristic of AB·CB chromosomes. This loss must be an important factor in the elimination of recombinant types and maintenance of the intact AB·CB inversion association in nature. (Work supported by grant from The National Science Foundation.)

STINSON, H. T., JR., Connecticut Agricultural Experiment Station, New Haven, Connecticut: *An alteration leading to change of a species-specific characteristic of the Oenothera chloroplast.*— In Oenothera, plastid mutation has been assumed for the origin of the several hereditarily distinct chloroplast types, distinguishable, when combined with identical nuclei, by their chlorophyll phenotypes and by their relative rates of multiplication. Oenothera plastid mutations thus far encountered, however, have been to defective, nongreen plastids. A recent unexpected chlorophyll variegated plant has suggested an alteration in which one plastid type has undergone modification to another plastid type in at least one characteristic. The female parent of the unexpected variegated plant was a solid colored yellow-green branch of the variegated F_1 hybrid *O. parviflora* ♀ × *O. argillicola* ♂. The yellow-green branch possesses *argillicola* plastids (*A*), contributed by the pollen, and the complex combination *α parviflora-*^{h}*argillicola* (*ap-a*; ·14). The male parent was *argillicola* (*A aa*). The cross *Aap-a* ♀ × *A aa* ♂ produces *Aap-a* plants, all of which should be yellow-green. One plant, however, was yellow-green with a normal green sector. Only *parviflora* plastids (*P*) are known to be green with *ap-a* nuclei; the green sectors must therefore contain *parviflora*-like plastids (P^1). But testcrosses show the P^1 plastids to be different from ordinary *P* plastids in rate of multiplication ($P^1 > P$). Results to date tend to eliminate nuclear events as responsible for the plastid alteration, and suggest mutation in an extranuclear component. A likely, but not necessarily the only, interpretation favors a change from *A* to *P* plastids in chlorophyll-forming capacity of the plastids with retention of one multiplication rate of *A* plastids, which are known to multiply at a faster rate than *P* plastids.

STORMONT, C., W. J. MILLER, and Y. SUZUKI, University of California, Davis, Calif.: *The convergence of the A-H and D systems of bovine blood groups.*—D is one of the more recently discovered blood factors in cattle. In the first report on D (STORMONT and SUZUKI 1957), it was assigned to a system by itself but subsequent data with improved D reagents have nullified that assignment. It is now found that A_1, A_2, D and H are all members of the same system, and we propose to call that system A rather than A-H.—The first clue relating D with A_1, A_2 and H came as result of typing 56 samples of blood from American buffalo (*Bison bison*) kindly provided by MR. JULIAN A. HOWARD, Manager of the Wichita Wildlife Refuge, Cache, Oklahoma. Only two A phenotypes were distinguishable in these 56 animals, namely A_1A_2D-positive and A_1A_2DH-negative. We have proposed elsewhere that these results can be explained by postulating a pair of phenogroups A_1D and "–" (the negative alternative) controlled, respectively, by bison alleles a^{A_1D} and *a*. The situation in *Bos taurus*, the homologous species, is much more complex. So far, no A_1A_2DH-negative cattle have been encountered. The cattle alleles presently indicated are a^{A_1}, a^{A_1D}, a^{A_1H}, a^{A_2D}, a^{A_1DH}, a^{D} and a^{DH}. By far the most frequent allele is a^{D}. The allele a^{A_1D}, equivalent in phenotypic effect to a^{A_1D} of bison, has so far been definitely established only in Aberdeen-Angus cattle.

STRICKLAND, W. N., Department of Biology, Stanford University, Stanford, Calif.: *Interference in short chromosome segments of Neurospora.*—In most organisms chiasma interference is positive when intervals are short but the situation with respect to chromatid interference is not clear. The LINDEGREN's (1942) and WELSHONS (1955) have obtained evidence that negative chromatid interference is present in short intervals. The present study was designed to test for chromatid interference across intervals which were short enough to avoid the confusion caused by multiple exchanges within an interval but long enough to obtain a reasonable number of multiple exchanges over the marked region. The centromere independent group V markers *hist-1* — 5 — *inos* — 5 — *bis*(B6) — 10 — *pab-2* were suitable. Strains *hist-1 bis*(B6) (320)a, *hist-1 bis*(B6) (324)a, *inos pab-2* (927)A and *inos pab-2* (72)A were crossed in all possible combinations. Seventy-five hundred tetrads have been obtained for analysis by a technique wherein shot ascospores are collected in groups representing unordered asci (STRICKLAND 1960). Chiasma interference was very strong ($C = 0.1 - 0.3$) in all the crosses. Significant evidence of chromatid interference was obtained with some parental combinations but not others. Different types of chromatid interference were obtained according to the parental combination and the distance between exchanges. The most striking example is nine 2-strand: one 3-strand: nine 4-strand double exchanges obtained from (320) × (927), with $P < 10^{-5}$. (Supported by a grant from the National Science Foundation.)

SWANSON, HAROLD D., (Introduced by E. H. GRELL), University of Tennessee, Knoxville, Tenn.: *A model for differentiation, based on gene inhibition by basic proteins.*—Studies of isolated cytoplasmic proteins of the rat disclosed a class of neutral basic proteins, varying in amount from tissue to tissue. Further investigation showed that these proteins could enter isolated nuclei and change the degree of expansion of the nuclear gel, probably by combining with DNA. These properties were not shared by serum proteins of similar charge. Consideration of these properties in light of cytological and embryological evidence indicates that these basic proteins may be agents of differentiation. A model of embryological differentiation, based on properties of specific known substances, is presented and discussed. It is proposed that specific basic proteins inhibit the activity of certain genes in any one tissue, producing a unique pattern of genic activity in each kind of differientiated cell. (This research was performed in the Biology Division, Oak Ridge National Laboratory, under the support of the Oak Ridge Institute of Nuclear Studies.)

TAKEBE, H., and P. E. HARTMAN, The Johns Hopkins University, Baltimore, Maryland: *An X-ray resistant mutant of Salmonella phage P22.*—At low doses of X-rays (250 kvp, 30 ma, 1 mm Al) in protective media, P22 is several times more resistant than T_2L to X-ray inactivation by predominantly direct effects. At X-ray doses above 20,000r, both T_2 and P22 phages suspended in T_2 buffer, distilled water or physiological saline exhibited approximately equal, rapid exponential declines (rate for T_2 twice rate at low doses). This inactivation is presumed to be due chiefly to indirect effect since nutrient broth or five times concentrated nutrient broth substantially protect. Whereas the inactivation curve for T_2 showed no break, a sharp break was noted in the P22 curve at about 10^{-5} survival. Isolation of survivors after prolonged X-irradiation in T_2 buffer provided an X-ray resistant mutant. The mutant was identical with P22 in its serological and plating characteristics. The mutant was about four-sixfold more resistant to X-ray inactivation in aqueous suspension than was wild type P22. It was also over fivefold more resistant to killing by preirradiated T_2 buffer than was wild P22. The resistant phenotype is clonally inherited and a stable property of the phage. Therefore we conclude that a mutant of P22, relatively (but not completely) resistant to indirect effects of X-rays, has been isolated.

TJIO, J. H., T. T. PUCK, and A. ROBINSON, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md., and Dept. of Biophysics, University of Colorado Medical Center, Denver, Colorado: *The human karyotype with special reference to the satellited chromosomes.*—Photographs will be presented showing the normal human somatic chromosomes, including their

arrangement in accordance with the new numbering system adopted by the international study group which convened in Denver in April, 1960. The presence of six satellited chromosomes, instead of only the four which had been first identified will be demonstrated. Abnormal satellites found in two patients with Marfan syndrome will be exhibited, and a series of photographs will be shown demonstrating satellite morphology of human chromosomes throughout prophase and metaphase.

TSAI, L. S., Yale University, New Haven, Conn.: *The developmental effects of bw^5 deficiency in Drosophila melanogaster.*—The mutant Df(2)bw^5, is a homozygous embryonic lethal. It is proved that this is a deficiency of a double band, 59E1, on the right arm of the second chromosome. Two faint bands, 59E3 and 59E4, may also be missing. Among the 25% embryos standing for the Mendelian class, 20% show retardation of cellular movement during head involution. The head fold of this embryo is incomplete. Accordingly, the frontal sac is incomplete, the corpus allatum of the ring gland is lacking. All chitinous structures in the head region are dislocated or deformed. Somatic muscular contraction is abnormal. The potentiality for growth and differentiation of these embryos was tested by transplantation into wild type larval hosts. It shows that the mutant implant's hypoderm is competent to attain a first larval moult but is unable to produce imaginal discs under the influences of the host hormone. The tissues of the mutant implant develop pycnotic nuclei and cytoplasmic vacuoles. The function of the mutant's ring gland was tested by transplanting several head pieces (including the ring gland) of the mutant embryo into normal adult male host. It indicates that the ring gland of the mutant implant does not produce functional moulting hormone in contrast with the control. The other 5% of the embryos within the Mendelian class appear to be morphologically normal, but their muscular contraction is subnormal and die within the egg cases. The implants of this embryo, however, are able to attain a second larval moult and to develop imaginal discs under the influences of the larval host's hormone. The results here suggest that the failure of the typical mutant embryo to produce normal hypoderm and imaginal discs when transplanted may be a consequence of the failure of the cells to attain their normal position during head involution.

TUVESON, R. W., and E. D. GARBER, Wayne State University, Detroit 2, Mich.; University of Chicago, Chicago 37, Ill.: *Experimentally induced alterations in nuclear ratios of heterocaryons of a phytopathogenic fungus, Fusarium oxysporum f. pisi.*—Spores of one genotype are produced by heterocaryons, grown on minimal medium, of diauxotrophic strains of *Fusarium oxysporum* f. *pisi.* Nuclei with these genotypes are called "dominant nuclei." The frequency of spores with a dominant nucleus is determined by supplementing the minimal medium with graded concentrations of the metabolite(s) synthesized by the dominant nucleus. In two heterocaryons, it was possible, by supplementation, to alter the frequency of spores with the dominant nucleus so that an increasing number of spores with the recessive nucleus was observed. By using appropriate concentrations of supplement, only spores with a recessive nucleus were isolated. The concentration of supplement required for complete reversal differed in each heterocaryon although the heterocaryons had a common, recessive nucleus.

VON BORSTEL, R. C., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *Death of Drosophila embryos by exchanges in the tandem metacentric X chromosome.*—Approximately 22% of the eggs from a female containing the tandem metacentric X of NOVITSKI and LINDSLEY are expected to have chromatid bridges attached to the pronucleus from one half the single and one half the three-strand double exchanges. Twenty-three females, TMX, $\frac{sc^{8L}X^{C2R.}X^{C2L.}+^{R}}{}$, were crossed with attached-XY males, $Y^{S}X.Y^{L}$, *In(1)EN, B y $w^{e}bb^{1}$*/Y. These produced 306 eggs, 147 of which hatched, and from which 81 *B y* males eclosed; thus it seems certain that nearly every egg was fertilized. Of the 159 unhatched eggs, 102 were fixed and stained. Fifty-three died with the abortive blastula appearance characteristic of nullo X, nullo Y embryos. Forty-six died during very early development. Correcting for the unex-

amined, unhatched eggs, the deaths during very early development amount to 23% of the total number of eggs; this is consistent with the frequency of eggs expected to have a chromatid bridge associated with the pronucleus.—Embryos dying during very early development exhibit a typical syndrome consisting of polyploid, disturbed, basket-shaped "metaphases" with chromosomes lying at random in the spindle. The "basket metaphase" syndrome is probably associated with induction and breakage of the second meiotic anaphase bridge. "Basket metaphases" also appear to be characteristic of Drosophila embryos dying very early in development after exposure to X-rays. (National Science Foundation Postdoctoral Fellow at the Istituto di Genetic, Univ. di Pavia, Pavia, Italy, on leave of absence from ORNL.)

Wallace, A. T., Agricultural Experiment Station, University of Florida, Gainesville, Fla.: *The frequency of induced mutations at a specific locus in oats.*—Seeds of the Victorgrain variety of oats, which are susceptible to the fungus, *Helminthosporium victoriae*, were irradiated with cobalt-60 gamma-rays and grown to maturity in isolation. The germinating seed in the X_2 generation were treated with toxin produced by the fungus, *H. victoriae*, and resistant seedlings selected. These resistant seedlings represent induced mutations at the locus controlling the reaction to the disease in the oat variety. When seeds equilibrated to 10% moisture were irradiated at 0, 9, 18, 27, and 36 kr at an intensity of 4.5 kr per minute, the induced mutation rates ($\times 10^{-8}$) were 0, 10.4, 13.6, 0.9, and 5.1 per roentgen per locus, respectively. When the seed moisture was 33%, obtained by hydration at 2°C, and irradiation applied immediately after hydration at 0, 4.5, 9, and 13.5 kr at an intensity of 4.5 kr per minute, the induced mutation rates ($\times 10^{-8}$) were 0, 35.9, 10.9, and 1.5 per roentgen per locus, respectively. When the seeds with 10% moisture were irradiated at 17 kr and 25 kr, at low intensities, the induced mutation rates ($\times 10^{-8}$) were 8.8 and 4.6 per roentgen per locus, respectively. The number of panicles tested per treatment varied, but ranged from 4100 to 9000. These were sufficient to give significant differences (90% confidence limits) in mutation rates between treatments in most cases. These induced mutation rates are in the same order of magnitude as those reported for mice and Drosophila; did not increase linearly with increasing dosage; were influenced by moisture content of the seed at the time of irradiation; and were not influenced by the range in intensity tested (20r/minute to 4.5/kr minute). (This work supported in part from AEC Contract No. AT-[40-1]-2562.)

Weijer, J., and E. S. Dowding, University of Alberta, Edmonton, Alberta, Canada: *Nuclear exchange in the heterokaryotic cell of Neurospora crassa.*—A heterokaryon of *Neurospora crassa* was established in culture between two macroconidial biochemical mutants; leucine (*leu-3*, linkage group I) and tryptophan (*tryp-3*, linkage group II). Conidia of this heterokaryon fertilized proto-perithecia of a wild type strain. Perithecia matured and dissection of ascospores from individual asci showed that very infrequently (1 per 1000 asci) segregation for the two biochemical mutants took place in one and the same ascus. The spermatial nucleus, therefore, carried the leucine mutation as well as the trytophan mutation. In all other asci dissected there was a normal segregation either for leucine or for tryptophan. Since only one nucleus functions as the spermatizing nucleus, it was assumed that nuclear exchange in the heterokaryotic cell could have brought about somatic recombination. Cytological investigation into the mitosis of Neurospora and Gelasinospora revealed that in both species the nuclei are straight or coiled filaments frequently coming together in tangled masses. The thread-like nature of the nuclei may have genetical implications, because during simultaneous mitosis within a multinuclear cell, nuclear components may become exchanged. (This study was aided by a grant of the National Research Council of Canada.)

Weir, J. A., University of Kansas, Lawrence, Kansas: *Use of crosses and double matings to evaluate male influence on the sex ratio of mice.*—A compact theory, vast literature, and paucity of experimental evidence have made the sex ratio of mammals a favorite subject for the dilet-

tante. Work to be described makes use of unique inbred lines of mice with sex ratios and color formulas as follows: pHH 52.8 ± 1.00, *ln/ln, a/a*; pHL 41.8 ± 0.93, *ln/ln, a^t/a^t, b/b*. New information on the male effect has been obtained by mating pHH and pHL ♂♂ to ♀♀ of four inbred lines (*inter se* sex ratios of the lines, based on 5007 mice, in paren.): C_3H (50.8), AKR (49.9), A/He (50.4), BALB/C (50.2). Sex ratios at birth of F_1 litters by pHH sires were: 53.9, 60.0, 60.8, 56.6; by pHL 42.5, 39.6, 38.0, 40.5.—Results from artificial inseminations have been extended under more natural conditions by means of double matings; paternity in each instance has been determined by color. Of the 147 young born in 21 litters of mixed paternity, there were 39♀♀:33♂♂ (45.8% ♂♂) from pHH sires and 48♀♀:27♂♂ (36.0% ♂♂) from pHL sires. Of 171 single sire litters, 74 were by pHH (52.2% ♂♂), 97 by pHL (38.6% ♂♂). Differential viability does not seem to exert any consistent influence on sex ratio. Effects of parity, presence or absence of plug, strain of female, heterosis, and behavior will be discussed. (Work supported by U. S. Public Health Service Research Grant CY 3655.)

WELSHONS, W. J., and E. S. VON HALLE, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *Further studies of pseudoallelism at the Notch locus of Drosophila.*—In previous publications concerned with the pseudoallelic nature of the Notch locus, a tentative map of the locus was presented. Additional experiments demonstrated the separability of loci which were given a tentative position in the earlier publications, and, in addition, at least one new mutant has been added to the pseudoallelic series. The linear order of pseudoalleles from left to right on the X chromosome is as follows: facet (*fa*), facet-notchoid (fa^{no}), $Notch^{264-40}$ (N^{264-40}), split (*spl*), $Notch^{j24}$ (N^{j24}), $Notch^{Co}$ (N^{Co}), and notchoid (*nd*). The locus consists of at least seven separable pseudoalleles, four of which are recessive visibles, and three which are dominant Notch mutants (recessive lethals). The various recessive visibles are sometimes complementary in phenotype, while combinations of the dominant Notch mutants, i.e., recessive lethals, are all noncomplementary. Pseudoallelic studies in Drosophila often show a poor correspondence with similar studies performed in microorganisms; however, the recessive lethal system at the Notch locus promises to show a striking similarity while the recessive visible system at the same locus does not. Perhaps the recessive lethals in Drosophila are more equivalent to the mutants ordinarily used in microorganisms. This in turn suggests that recessive visibles in Drosophila might be more nearly equivalent to the leaky mutants found in lesser organisms. Since leaky mutants are not ordinarily utilized, the comparison of recessive visible pseudoalleles in Drosophila with pseudoallelic systems in microorganism would be less exact.

WOODWARD, V. W., and WILDA COOK, Wichita University, Wichita, Kansas: *Genetic vs. complementation mapping of the pyr-3 region in Neurospora.*—A detailed genetic map of the *pyr-3* region of linkage group IV of *Neurospora crassa* has been presented (SUYAMA, Y. *et al.*, Genetica XXX (1959):293–311). Even though the various sites show additivity and linearity, there is no evidence that reciprocal chromosome exchange obtains. The mechanism providing the "recombinants" being obscure, it seemed worthwhile to compare the genetic map of the *pyr-3* locus with a map based on heterocaryon complementation.—The genetic map of the *pyr-3* region is based on crosses of 32 independently isolated mutants, in all possible combinations. The same mutants, plus 20 additional ones, were used in the heterocaryon tests. Attempts to force heterocaryons were made both in liquid minimal medium and on minimal agar plates.—Data will be presented that illustrate the division of the *pyr-3* region into six cistrons. (The region includes 13 mutons, excluding multisite mutations.) Five of the cistrons extend a map distance of two mutons, while the sixth extends a distance of three mutons. According to the map distances derived from breeding tests, the shortest cistron is 0.0014 map units, and the longest is 0.0040 map units in length. The surprising aspect of the relationship is that the distances between cistrons include the shortest distances measured by the breeding tests, i.e., 0.0007, 0.0008, 0.0006, and 0.0011 map units.—Discrepancies between the genetic and the complementation maps do appear. Mutants 7, 8, and 10 are homoallelic according to the genetic map, yet 10 shows positive complementation with both 7 and 8 (7 × 8 is negative). Mutants 3 and 46, which likewise

are homoallelic, complement to a small degree. The interpretation of these results is difficult, but they do indicate that homology between the two methods of mapping short regions of chromosomes is not absolute. (This research was supported by the National Institutes of Health, Grant no. RG-5833(C2), and the American Cancer Society, Kansas Division.)

YERGANIAN, GEORGE, The Children's Cancer Research Foundation and Harvard Medical School, Boston, Massachusetts: *"Brittle-bristle," genetic evidence for the Triheterosomic scheme of sex determination."*—A peculiar hair condition, "brittle-bristle," limited to female Chinese hamster, *Cricetulus griseus*, was first reported in these Records by YERGANIAN, MCKAY and by GAGNON in 1957. Analysis of 20 litters of the BUY strain indicates this mutation to display a reciprocal inheritance pattern to that described for classic sex-linked genes. In the case of "brittle-bristle," the male is the *carrier* and only female offspring express this condition.—Morphological and physiological observations reveal a dual nature of the X chromosomes in females in the form of a "Predominant X_1" and a "Retarded X_2," in contrast to assumed homology or classic pairs of X's. Since the male has a "Predominant X_1" and classical Y chromosome, the gene for "brittle-bristle" is considered to reside in the short arm of the X chromosome and is expressable *only* when the mutant is in the $X_2{}^b$ state. The X_2 chromosome is considered to differentiate to the X_1 phase, when an X_2-bearing egg is fertilized by a Y-bearing sperm to give rise to a normal X_1Y male.—The short arm is altered physiologically from "heterochromatin" to "euchromatin" and, as a result of this complex function, X_2-mutant expressions are masked ($X_1{}^{(b)}$) in the male. Thus the male is the carrier for this peculiar sex-limited and X_2-linked expression. The mutation is neither dominant nor recessive because of the masking phenomenon. The Triheterosomic scheme for sex determination ($X_1X_2{:}X_1Y$) is a well-established entity in the Chinese hamster. Trials with normal and intersex human cell strains are currently underway.

RADIOSENSITIVITY AT SPECIFIC AUTOSOMAL LOCI IN MATURE SPERM AND SPERMATOGONIAL CELLS OF DROSOPHILA MELANOGASTER[1]

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THE characteristics of mutational damage recovered from spermatogonial cells are important for considerations of genetic risks from radiation. The genetic and cytological types of mutations recovered from spermatogonia determine the chances of genetic damage offered the ensuing populations. To study these characteristics, radiation studies were carried out in Drosophila where well defined genetic and cytological tests are available. The previous study showed a higher mutation rate for mature sperm than for spermatogonia (ALEXANDER 1954). Different total doses were used, and tests for mature sperm given 900r of X-rays were not available to clarify whether sperm rates were significantly higher when calculated from only nonbreakage types. Additional data were obtained for spermatogonial cells treated with 900r, and genetic and cytological data were obtained for mature sperm treated with 900r. Direct comparisons of genetic damage in the two types of cells are made.

EXPERIMENTAL PROCEDURE

The detailed procedures appear in a previous paper (ALEXANDER 1954). Only the general procedure will be outlined here. Mature sperm were treated in three-day old males of the Oregon-R, Oak Ridge Strain of *Drosophila melanogaster*. For spermatogonial tests, 20–24 hours old larva were irradiated. Both mature males and larva were treated with 900r of X-radiation using a Maxitron Unit, operating at 250 kvp, 15 ma and using a ½ mm cu, 1 mm Al filter, at a rate of approximately 85r/minute at a distance of 84 cm from the tube.

In the mature sperm tests, males were mated to females homozygous for eight recessive genes (res complex) on the third chromosome. These are *ru* (roughoid), 0.0; *h* (hairy), 26.5; *th* (thread), 43.2; *st* (scarlet), 44.0; p^p (peach), 48.0; *cu* (curled), 50.0; *sr* (stripe), 62.0; e^s (sooty), 70.7. The F_1's were checked for mutations at any of the eight loci. For the spermatogonial tests, adult males treated as larvae were mated, individually, to one or two res females. Variants appearing among the F_1's from sperm and spermatogonia were tested genetically.

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Genetic mutations were tested for viability by dihybrid crosses of heterozygotes containing the induced mutation and either e^{11}, p^{p} or *In(3R)C, Sb e l(3)e*. Control tests were run for each radiation test. Cytological examinations were made by the usual aceto-orcein squash preparations.

RESULTS

In mature sperm, 23 mutations were recovered from 54,683 flies. An average mutation rate of 5.82×10^{-8} per r/locus is obtained by dividing the number of mutations by the total number checked, number of loci and the dose. In the viability tests (Table 1), 13 or 57 percent of the 23 mutations were viable as homozygotes. Only six showed normal viability when the percentage of homozygotes of induced mutations was compared to the 25 percent expected from dihybrid crosses. In seven, viability was reduced to approximately one half the expected values. The remaining ten mutants (43 percent) were semilethal (3) or lethal (7). No homozygotes were recovered from mutants of the lethal class; less than one percent of the expected homozygotes were recovered from semilethals. Cytological analyses of mutants produced in mature sperm showed 18 with no detectable cytological changes (point mutations), two with inversions, two with deletions and one with a complex translocation.

In spermatogonial tests, 17 independent, "cluster" mutations have been recovered from 2,832 males that were treated in early larval stage. A total of 423,-

TABLE 1

Genetic and cytological analyses at specific autosomal loci

	Viability tests			
	Homozygous viable		Homozygous lethal, semilethal	
	Percent	Number	Percent	Number
Mature sperm				
3000r*	34	20	66	38
900r	57	13	43	10
Spermatogonia				
900r	53	8	47	7

	Cytological analyses			
	Point mutations		Chromosome aberrations	
	Percent	Number	Percent	Number
Mature sperm				
3000r*	54	30	46	26
900r	78	18	22	5
Spermatogonia				
900r	100	16	0	0

* Alexander 1954; Ward and Alexander 1957.

536 F_1's were checked for these tests. The loci of the mutations and the size of the "cluster" of mutations (mutants/total sample) are as follows:

h	*st*	p^p	*cu*	*sr*	e^s
5/598; 17/770	3/142	1/139	1/225;4/297 1/228;3/153 1/202	2/161;1/342 2/181;1/281	31/469;1/110 18/388;1/240

Mutations have been recovered at six of the eight loci tested. No mutants have been recovered at the *ru* or *th* loci. These loci also showed low mutation rates in the sperm tests. The viability and cytological data for spermatogonia are included in Table 1. Both viable mutations (33 percent) and lethal or semilethal mutations (47 percent) have been recovered from spermatogonial cells. Cytological examination of the mutations showed an absence of chromosome aberrations.

A total of 99,703 individuals (Oregon-R, Oak Ridge Strain) have been checked in control tests. Only one possible spontaneous mutation, a slight ebony allele, has been recovered, and it is so doubtful that no corrections for it were made in the radiation tests.

DISCUSSION

The recovery of a number of identical mutations from the same male is characteristic of radiation damage in spermatogonial cells. The ratio of the number of mutants to normal flies varies depending upon the number of spermatogonia present at time of treatment. The estimates made from the genetic data of 8–171 cells with an average of 68 agree, in general, with the 16–76 cells reported by SONNENBLICK (1941) from histological examinations.

Mutation rates in spermatogonia are estimated by obtaining means for the rates calculated from the progeny of each male (ALEXANDER 1954). Since most males contain no mutations, the expression for the mean rate becomes: $1/2{,}832\ (5/598 + 17/770 \text{——} 1/240) = .9191 \times 10^{-4}$. An average mutation rate of 1.28×10^{-6} per r/locus is obtained by dividing the mean rate by the total number of loci (8) and the dose of 900r. The 95 percent confidence limits for the 17 spermatogonial mutations are ten and 27 (MOLINA 1942). Expressing the average rate in these terms the limits of the rate become $(0.75\text{–}2.03) \times 10^{-6}$ per r/locus. These limits agree with those for previous calculations that were based on fewer mutations. Since the observed rate in Drosophila is essentially the same, the estimate of a mutation rate 15 times higher for mouse spermatogonia than for Drosophila spermatogonia is still valid when testing with similar dose rates (RUSSELL 1956).

A comparison of sperm and spermatogonial rates necessitates a consideration of the cytological data since chromosome aberrations have not been recovered from treated spermatogonia (Table 1). Only point mutations in the two types of cells should be compared. The 95 percent confidence limits for the 18 point mutations from mature sperm are 10.5 and 28.5. The limits of the average rate

of (2.66–7.23) $\times 10^{-8}$ per r/locus show no overlap with the 95 percent confidence limits of (0.75–2.03) $\times 10^{-8}$ per r/locus for spermatogonia. These data show that the higher mutation rate in mature sperm is due, in part, to a difference in genetic mutability in the two types of cells. Since the rates were calculated with point mutations (nonbreakage types), selection of chromosome breakage from spermatogonia cannot account for the entire difference in the rates. The low mutability of spermatogonia, the possible failure of chromosome reattachment, and selection against breakage types in mitotic and meiotic divisions indicate a low recovery rate of chromosome aberrations.

The proportions of viable, lethal and semilethal mutations were similar in mature sperm and spermatogonial cells with 900r of X-radiation. In mature sperm, one half of the lethal mutations were associated with chromosome aberrations; whereas, in spermatogonia all lethals were point mutations. In mature sperm, a greater proportion of lethals and semilethals resulted when the dose was increased to 3000r. The increase in lethals is accompanied by an increase in chromosome breakage as reflected in the larger number of chromosome aberrations. The differences in point mutations and chromosome aberrations at the two doses are similar to those obtained by OLIVER (1932) for sex-linked recessive mutations.

SUMMARY

The mutation rate, when calculated with point mutations, was higher in mature sperm than the rate for spermatogonia with 900r of X-radiation. The higher rate in sperm shows a difference in the mutational sensitivity of the two types of cells. Selection of chromosome breakage types from spermatogonia cannot account for the difference. The spermatogonial mutants show the same proportion of viable and lethal mutations as obtained with mature sperm with 900r. The absence of chromosome aberrations remained characteristic of spermatogonial mutants.

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EVIDENCE FOR THE GENETIC CONTROL OF PHOTOREACTIVATION[1]

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PHOTOREACTIVATION is the reversal by near-ultraviolet or visible light of ultraviolet radiation damage to biological systems (JAGGER 1958). This phenomenon has been demonstrated in the intact cell in a spectrum of organisms (extending from bacteria to mammals) (JAGGER 1958) and *in vitro* with ultraviolet-inactivated transforming factor (deoxyribose nucleic acid) of *Hemophilus influenzae* (GOODGAL, RUPERT, and HERRIOTT 1957; RUPERT, GOODGAL, and HERRIOTT 1958). Photoreactivation of the above factor following ultraviolet treatment proceeds only in the presence of cell-free extracts which thus far may be obtained from either *Escherichia coli* (RUPERT *et al.* 1958) or baker's yeast (RUPERT, in press). The activity of these extracts resides in a cellular component, probably an enzyme, present in some organisms while absent in others. One implication of the above *in vitro* experiments, when coupled with the observed variability or lack of photoreactivation within species or families of microorganisms (BELLAMY and GERMAIN 1955; STUY 1956; GOUCHER and KOCHOLATY 1957; GOODGAL *et al.* 1957), is that the photoreactivation mechanism in the intact organism is under genetic control. Direct evidence for the genetic control of photoreactivation is presented in the present study on the photoreactivation of ultraviolet-induced extrachromosomal mutations (producing respiratory deficiency) in yeast.

Genetic nomenclature of ultraviolet-induced respiration deficient mutants: The induction of respiration deficient mutants of yeast by ultraviolet and the photoreactivation of this mutation have been previously described (RAUT 1954; PITTMAN 1956; SARACHEK 1958; PITTMAN, RANGANATHAN, and WILSON 1959). These mutants are phenotypically similar to spontaneously derived respiration deficient variants in their inability to (1) consume significant quantities of oxygen (WHELTON and PHAFF 1947), (2) grow on an obligate aerobic substrate such as lactate (OGUR and ST. JOHN 1956), and (3) reduce triphenyl tetrazolium chloride (OGUR, ST. JOHN, and NAGAI 1957). Genetic analyses (RAUT 1954; PITTMAN 1959) show that the majority (about 95 percent) of respiration deficient mutants derived from ultraviolet irradiated haploid stocks fall into three classes, each having been previously described by EPHRUSSI and co-workers (CHEN, EPHRUSSI, and HOTTINGUER 1950; EPHRUSSI 1953): (1) petite mutants, the principal class, which result from the loss or functional inactivation of extrachromosomal determinants; (2) genic (or segregational) mutants which carry the recessive allele of a gene controlling respiration; and (3) double mutants

[1] This work has been supported by grants from the United States Atomic Energy Commission and the Damon Runyon Memorial Fund for Cancer Research, Inc.

which carry both of the above defects. Genic and double mutants are rarely encountered (less than one percent) among irradiated survivors and only after high doses of radiation; hence, their frequency does not significantly bias the mutation-dose curves (Figure 3a) generally used to describe the induction of petite mutants. Double mutants have not been detected spontaneously in our normal stocks over a period of three years, although they occur with a spontaneous frequency of about five percent in most genic stocks. The remaining (about five percent) mutants induced by ultraviolet consist of heterogeneous colonies which usually revert to wild type on subculturing (PITTMAN 1959).

Descriptive genetic analyses of the petite, genic, and double mutants are presented elsewhere (CHEN *et al.* 1950; RAUT 1954; PITTMAN 1959), and only the genetic nomenclature employed in the present study need be considered: Normal stocks and the three mutant types are designated: $r^+\gamma^+$ (normal); $r\,\gamma^+$ (genic); $r^+\gamma$ (petite); and $r\,\gamma$ (doubly defective), where r^+/r denote the gene pair controlling respiration, and γ^+/γ denote the presence (absence) of the extrachromosomal factor. Use of the latter symbols is justified on the grounds that firstly, the extrachromosomal factors appear to carry their own genetic information (EPHRUSSI 1958; PITTMAN 1959), and secondly, the defect may reflect a functional inactivation, rather than physical loss, of the extrachromosomal factor.

Method for distinguishing the three types of respiration deficient mutants: The above three mutant types are phenotypically indistinguishable when oxygen consumption, growth on lactate, and reduction of tetrazolium chloride are employed as the diagnostic tests for respiratory deficiency (PITTMAN 1959). The mutants can be readily identified, however, by the method outlined in Figure 1. This method utilizes the fact that when the three mutant types are crossed in all combinations on glucose nutrient agar, only diploids of $r^+\gamma \times r\,\gamma^+$ constitution are respiration sufficient and capable of growth on lactate, the hybrids of the remaining combinations being respiration deficient and incapable of growth on

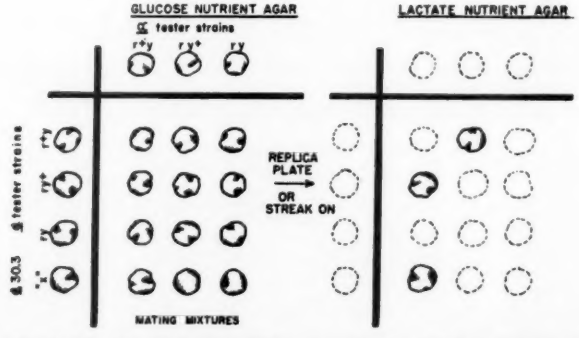

FIGURE 1.—Method for identifying the different respiration deficient mutants. a/a denote the mating type gene pair; r^+/r, the gene pair controlling respiratory competence; and γ^+/γ, the presence (absence) of the functional extrachromosomal factor. Dotted lines designate the absence of growth; closed lines, luxuriant growth.

lactate. Thus, to classify mutants of unknown constitution, a sample of each mutant is first "spotted" on glucose nutrient agar and then mixed individually with $r^+\gamma$, $r\,\gamma^+$, and $r\,\gamma$ tester strains of appropriate mating type, as shown in Figure 1. After 24 hours, the mating mixtures (containing about 30 percent diploids) are replica plated, along with the parents, on lactate nutrient agar. A mutant may be classified on the basis of which (if any) hybrid grows on lactate (for example, mutant 30.3—"x" in Figure 1—was diagnosed as $r\,\gamma^+$). Alternatively, the above glucose plates may be overlaid with triphenyl tetrazolium agar to determine the presence of respiration sufficient hybrids. A team of two workers can classify several hundred mutants within a week by this procedure. It is evident that the method also provides a means whereby new mutants may be uncovered, and we have thus far uncovered two nonallelic mutants in some 3,000 colonies analyzed. (The general application of this method and its limitations with respect to "suppressive" mutants will be discussed elsewhere).

Origin of extrachromosomal mutants: Respiration deficient genic mutants are of singular interest since it can be shown with these stocks that the extrachromosomal factor associated with normal cells is transmitted in the presence of the recessive allele of the r gene and, consequently, independently of the inherent ability (inability) of the cell to respire. In 17 parallel plating experiments of a normal culture (8256) and a genic mutant (8256.27), 5.3 percent (181/3398) of the colonies arising from the former culture were $r^+\gamma$ and 8.3 percent (277/3328) of the colonies arising from the latter culture were $r\,\gamma$. These results and those obtained with 20 of 22 additional ultraviolet induced mutants indicate that the mutant frequency is slightly higher in genic strains than in the respective parental stocks. Transmission of the extrachromosomal trait in genic stocks has been previously noted by CHEN *et al.* (1950), and our studies simply serve to extend their findings. The essential point is that extrachromosomal mutants may be either $r^+\gamma$ or $r\,\gamma$, depending on whether they originate from normal or genic stocks respectively.

Preliminary experiments indicated that extrachromosomal mutants were induced with comparable frequencies in normal and genic stocks irradiated with intermediate doses of ultraviolet (20 percent survival). However, in contrast to previous results with normal stocks, the mutation in genic stocks was nonphotoreactivable. This phenomenon was studied quantitatively by a method permitting a direct enumeration of extrachromosomal mutants in both normal and genic stocks.

MATERIALS AND METHODS

Stocks: The principal cultures employed were adenine-dependent (*ad-1*) haploids 8256 ($r^+\gamma^+$), 8282 ($r^+\gamma^+$), 8256.1, 8256.27, 8256.30, 8282.1 (all $r\,\gamma^+$ mutants derived by ultraviolet irradiation of the preceding parental stocks); adenine-independent (*AD*) haploids 18971 ($r^+\gamma^+$) and 18971.2 ($r\,\gamma^+$); and $r^+\gamma$, $r\,\gamma^+$, and $r\,\gamma$ haploid "tester" strains of α and a mating types. The spontaneous frequency of extrachromosomal mutants in the above stocks never exceeded eight percent in experiments carried out over a two-year period.

Media: Glucose nutrient agar contained: peptone, 3.5 gm; KH_2PO_4, 1 gm; $(NH_4)_2SO_4$, 1 gm; $MgSO_4 \cdot 7\ H_2O$, 1 gm; Difco dried yeast extract, 5 gm; dextrose, 10 gm; agar, 25 gm; and distilled water, 1000 ml. Lactate nutrient agar was prepared by substituting 18 ml of 50 percent sodium lactate for the glucose in the above recipe. Tetrazolium overlay agar was prepared as previously described (OGUR *et al.* 1957).

Irradiation and plating procedure: Cultures were grown aerobically for five days on glucose nutrient agar, harvested, washed three times in M/15 KH_2PO_4, and resuspended in this menstruum to a concentration of 10^6 cells/ml. Thirty ml of the suspension were transferred to a petri dish bottom and exposed to ultraviolet radiation (95 percent 2537 Å) delivered from a Hanovia quartz envelope lamp housed in a dark chamber and centered 56 cm above the surface of the cell suspension. Radiation was delivered at a rate of 5.1 ergs/mm^2/sec at the surface of the cell suspension, as determined by a Westinghouse photometer. A "Solar" constant voltage transformer was employed to minimize fluctuation in the line voltage. A magnetic stirrer was employed to insure homogeneity of cell suspensions during irradiation. Control and irradiated samples were diluted when necessary, and appropriate aliquots (100–125 viable cells) spread on the surfaces of glucose nutrient agar plates. From ten to 20 plates were employed in each experiment at each dosage level. Plates were incubated for 3–4 days, after which colony counts were made and the number of the mutants scored. In photoreactivation (PR) experiments, dilution tubes (200 mm × 150 mm) containing control and irradiated suspensions were slanted on a rotating platform centered nine inches below a 500 watt (3200 K̊) projection bulb in a Fresnel photospot lamp. A glass trough containing one inch of 0.2 N $CuSO_4$ was interposed four inches below the spot lamp. The temperature during this treatment was maintained at 30°C (± 1°C) by the addition of 0.2 N $CuSO_4$ ice cubes to the trough and by an electric fan. Yellow light was employed throughout the experiments to facilitate pipetting and plating. Under the above conditions, maximum reversal of both killing and mutation was obtained with 60 min of "reactivating light" (hereafter designated as "PR light").

Mutation frequencies and survival ratios are based on from three to six independent experiments involving from 1000 to 5000 colonies at each dosage level. Approximately 95 percent of the mutants derived from irradiated normal stocks exhibited the stable respiration deficient phenotype on successive transfers on glucose; the remainder produced variegated or sectored colonies when overlaid with tetrazolium agar and were not included in calculation of mutant frequencies.

Conventional manometric procedures were employed for the determination of respiration and aerobic fermentation of cells. The reaction vessels contained 2.2 ml of yeast suspension (5×10^7 cells/ml); 0.5 ml of 6 percent glucose (or phosphate buffer) in the side arm; and 0.3 ml of 10 percent KOH (or buffer) in the center well.

Direct enumeration of extrachromosomal mutants in normal and genic stocks: The rationale of the method for scoring extrachromosomal mutants in normal and genic respiration deficient stocks is based on our own and previous observa-

tions (REAUME and TATUM 1949; TAVLITZKI 1951) that adenine-dependent (*ad-1* or *ad-2*) respiration sufficient yeast form a pink-red pigment when streaked on one percent glucose nutrient agar, whereas the petite (r^+y) mutants derived from these strains form little or no pigment on the same medium. In the present studies, we observed that genic ($r\ y^+$) mutants derived from pink *ad-1* stocks consistently produce a light brown pigment on one (or two) percent glucose nutrient agar. When normal and genic stocks were plated on the above medium, from one to eight percent of the colonies were white. Although phenotypically indistinguishable, the white colonies derived from normal and genic stocks were genetically different, as shown by crossing each with the three respiration deficient "tester" strains (Table 1). Of 842 mutants tested, those derived from normal stocks produced wild type diploids when crossed to $r\ y^+$ mutants *only*, whereas those mutants derived from genic stocks produced no respiration deficient diploids when crossed to each of the three tester strains. The white colony mutants derived from normal and genic stocks were therefore r^+y and $r\ y$, respectively. The genetic constitution of the white mutants from each culture was confirmed by tetrad analysis (PITTMAN 1959), the results of which are in accord with previous genetic studies (CHEN *et al.* 1950) of petite and genic mutants.

The possibility that the white mutants carried not only an extrachromosomal defect but also one or more recessive alleles of adenine genes (other than *ad-1*) controlling pigmentation (ROMAN 1956) was tested by crossing each of ROMAN's adenine (*ad-1–ad-7*) mutants to the following cultures: parental stocks 8256 *ad-1* and 8282 *ad-1;* ten spontaneous and ten ultraviolet derived white isolates of the preceding parental stocks; ten spontaneous and ten ultraviolet derived white isolates of genic mutants 8256.27 and 8282.3. On the basis of ROMAN's "spot" test for allelism (ROMAN 1956), all of the 80 white mutants and their respective parents were found to carry a single recessive gene allelic to the *ad-1* mutant of ROMAN's strains. Consequently, it may be inferred that the majority of

TABLE 1

The respiratory phenotype of diploids derived by crossing spontaneous and ultraviolet derived white colony isolates of normal (r^+y^+) *and genic* ($r\ y^+$) *stocks to respiration deficient tester strains*

Number of isolates tested	Origin	Crossed to tester strains: r^+y	$r\ y^+$	$r\ y$	Inferred genetic constitution of isolates
26 white	8256 (r^+y^+)-s	—	+	—	r^+y
425 white	8256 (r^+y^+)-UV	—	+	—	r^+y
29 white	8256.27 ($r\ y^+$)-s	—	—	—	$r\ y$
183 white	8256.27 ($r\ y^+$)-UV	—	—	—	$r\ y$
78 white	8282.3 ($r\ y^+$)-UV	—	—	—	$r\ y$
101 brown*	8256.27 ($r\ y^+$)-stock	+	—	—	$r\ y^+$

+ (—) = Respiration sufficient (respiration deficient).
s = Spontaneous.
UV = Ultraviolet derived.
* Parental type (brown) colonies of 8256.27 $r\ y^+$ picked at random.

white mutants arising spontaneously and following ultraviolet radiation carried the *ad-1* genotype of their respective parents.

Back mutants at the *ad-1* locus form white rather than pigmented colonies; however, their frequency was less than one per 10^6 *ad-1* cells plated (with or without radiation) on adenineless-synthetic agar. These back mutants would be expected to comprise a negligible proportion of the number of spontaneous and induced white colony mutants, and experimentally no back mutants were detected in over 4000 ultraviolet derived white colony mutants replica plated on adenineless-synthetic agar.

The concomitant loss of pigmentation and the extrachromosomal factor in the above adenine-dependent cultures permitted a direct enumeration of extrachromosomal mutants present in normal and respiration deficient genic stocks. In most experiments, however, the genetic constitution of the white mutants was confirmed by crossing from 30 to 100 white isolates to each of the three respiration deficient tester strains.

RESULTS

Figures 2a and 2b present the ultraviolet survival curves of normal (r^+y^+) and mutant (r^+y, $r\,y^+$, and $r\,y$) cultures plated without photoreactivation (D) and after maximum photoreactivation (PR). The percentage PR was approximately the same for each culture, although the mutants were generally more sensitive to killing than normal cells when plated in the dark. From these and previous results (PITTMAN and PEDIGO 1959), it would appear that the respira-

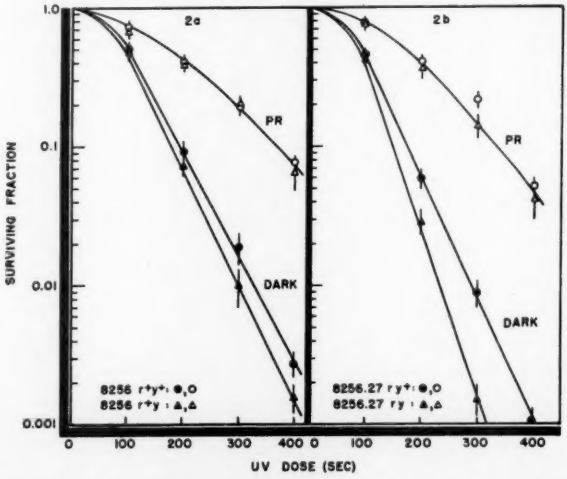

FIGURE 2.—Ultraviolet survival curves of normal (r^+y^+) yeast cells and of the three respiration deficient mutants, with and without maximum photoreactivation. (2a) normal (r^+y^+) and petite (r^+y) stocks; (2b) genic ($r\,y^+$) and double mutant ($r\,y$) stocks.

tory enzymes and cytochrome complement present in normal cells but lacking in the mutant type (SLONIMSKI and EPHRUSSI 1949) play no obligate role in photoreactivation of killing in yeast. Similar studies (LATARJET and BELJANSKI 1956) have shown that ultraviolet inactivation of a cytochrome and porphyrin deficient mutant of *E. coli* is photoreactivated to the same degree as that of the normal, wild type strain.

Characteristic dose-response curves for extrachromosomal (r^+y) mutants induced in normal cultures plated without photoreactivation and after maximum photoreactivation are shown in Figure 3a. The mutant frequency was reduced by roughly one half by PR light for doses greater than 100 sec. Since inactivation is photoreactivated to the same degree in both normal and r^+y cultures (Figure 2a), and since the r^+y mutant is not differentially killed by PR light (PITTMAN *et al.* 1959), the reduction in mutant frequency by PR light can be ascribed to the reversal of the mutational process producing the extrachromosomal defect.

The corresponding dose-response curves for extrachromosomal ($r\,y$) mutants induced in the genic stock plated without PR and after maximum PR are shown in Figure 3b. No significant decrease in mutant frequency in genic stocks occurred following PR light treatment. The mutant frequency (with or without PR) increased, however, more rapidly after 300 sec of ultraviolet than did the mutant frequency in normal stocks (Figure 3b). The similarity of the curves in Figure 3b cannot be ascribed to differential PR of killing in genic and $r\,y$ cells since the survival curves for both types following PR were essentially the same

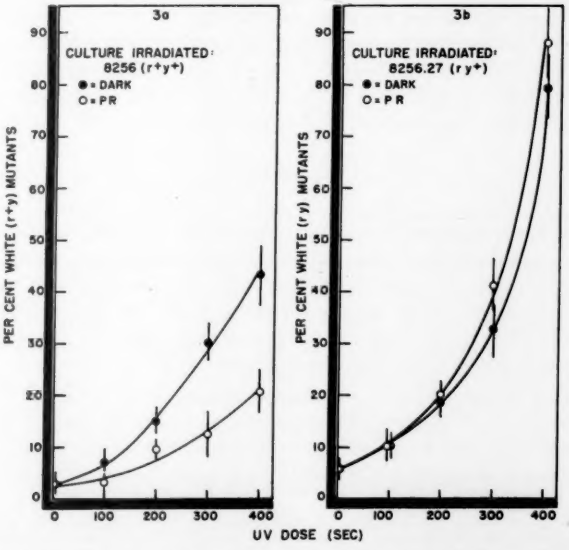

FIGURE 3.—Ultraviolet mutation-dose response curves for extrachromosomal mutations induced in normal (r^+y^+) and genic ($r\,y^+$) stocks, with and without maximum photoreactivation.

(Figure 2b). Similar results (summarized in Table 2) were obtained with four additional genic mutants, including one adenine-independent culture (18971.2 $r\,y^+$). The mutant frequencies given for the latter culture and its parents (18971 r^+y^+) were determined by crossing a minimum of 250 mutants to each of the three respiration deficient tester strains. It is evident that lack of photoreactivation of the extrachromosomal mutation is a characteristic of $r\,y^+$ stocks, whether genotypically *AD* or *ad-1*.

Previous experiments had shown that PR mutation curves, obtained with 0.2 N, 0.4 N, and 0.8 N $CuSO_4$ as the filter, were not statistically different. PR survival curves, however, were slightly higher with 0.2 N $CuSO_4$, although this concentration permits passage of considerable infrared radiation. A repeat of the above experiments with r^+y^+ and $r\,y^+$ stocks confirmed the above results and show that the difference in the photoreactivable trait of the strains cannot be ascribed to the 0.2 N $CuSO_4$ filter routinely used. Although the shape of the mutation curves, with and without PR, changes with ageing of cultures (PITTMAN 1959), we observed no photoreactivation of mutation in $r\,y^+$ stocks grown aerobically for 1, 3, 6 and 8 days prior to irradiation. (The authors are indebted to DR. B. RANGANATHAN for aid in many of the above experiments which, although not tabulated here, are available on request.)

The possibility that the observed lack of photoreactivation of the extrachromosomal mutations in genic mutants reflected a differential killing by PR light of genic and $r\,y$ mutant cells could not be demonstrated with singly or artificial mixtures of the two cell types exposed to PR light for as long as 110 minutes. Furthermore, no significant decrease in mutant frequency in irradiated genic stocks was noted in photoreactivation dose-rate experiments (Figure 4) extending over a period of 100 minutes. Under similar conditions, the mutant frequency in normal stocks, after a short lag, dropped sharply with increasing dose of light until maximum PR was obtained (Figure 4). It is concluded, therefore, that the

TABLE 2

Photoreactivability of the mutational damage producing extrachromosomal mutants in three normal (r^+y^+) *haploid stocks and four ultraviolet derived genic* ($r\,y^+$) *mutants*

Culture	Mutant induced	Percent mutants* Dose† Zero D	Zero PR light	300 sec. D	300 sec. PR light
8256 r^+y^+	r^+y	0.66	1.98	36.1	15.1
8282 r^+y^+	r^+y	0.47	0.45	39.5	12.5
8256.1 $r\,y^+$	$r\,y$	0.93	0.92	41.9	36.3
8256.30 $r\,y^+$	$r\,y$	2.57	2.72	45.6	58.2
8282.3 $r\,y^+$	$r\,y$	0.81	0.70	48.5	52.5
18971 r^+y^+	r^+y	1.01	1.21	39.2	13.6
18971.2 $r\,y^+$	$r\,y$	0.69	0.66	34.1	38.5

* Mutant frequencies for each culture are based on a minimum of 2200 colonies, except for stocks 18971 and 18971.2 (see text).

† Ultraviolet dose, 1.5×10^3 ergs/mm^2; D, without photoreactivation; PR light, after exposure to photoreactivating light for 60 min.

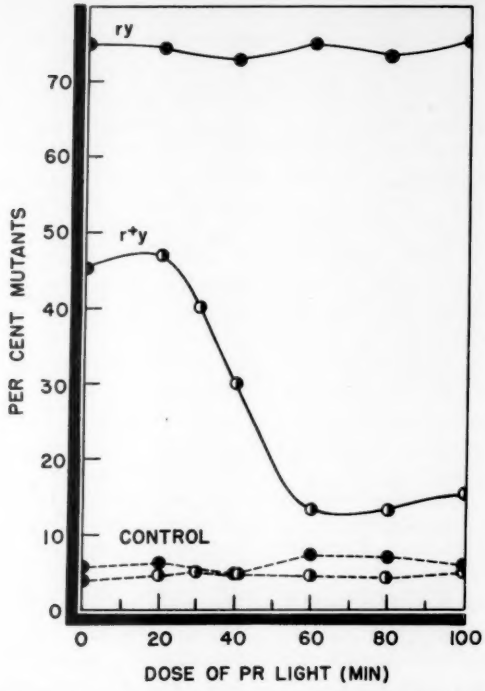

FIGURE 4.—Photoreactivation of extrachromosomal mutations induced in normal (r^+y^+) and genic ($r\ y^+$) stocks. Cells were irradiated for 400 sec and then exposed to PR light for the time intervals shown.

extrachromosomal mutation is photoreactivated in normal stocks but not in the genic mutant.

Photoreactivation of the extrachromosomal mutation in diploids homozygous dominant, heterozygous, and homozygous recessive for the *r* gene pair is shown in Table 3. In these experiments, four day-old cultures were harvested, washed, and irradiated with 300 (or 400) sec of ultraviolet and plated with and without exposure to PR light (60 min). PR light reduced the mutant frequency in the homozygous dominant (A) and heterozygous (B) diploids, but had no effect on the mutant frequency in the homozygous recessive (C) diploids. The genetic constitution of the mutants derived from the above diploids could not be determined since they were incapable of either sporulation or mating, the latter defect presumably due to heterozygosity for the mating type gene. However, similar studies (PITTMAN, COKER and PERRY, unpublished data) with diploid yeasts (strains 11294 and 11296) capable of mating with the three respiration deficient tester strains indicate that the majority of respiration deficient mutants induced by ultraviolet in diploid and polyploid yeasts result from extrachromosomal

TABLE 3

Photoreactivability of the ultraviolet damage producing extrachromosomal mutations in diploids homozygous dominant (r^+r^+), *heterozygous* (r^+r), *and homozygous recessive* (r r) *for the gene controlling respiration and of the segregants of four tetrads of heterozygote* (*B*) *and one tetrad of the dominant homozygote* (*A*)

Culture	Mutant induced	Dose*							
		Zero				300 sec			
		Dark		PR light		Dark		PR light	
Diploids	Exp:	1	2	1	2	1	2	1	2
(A) 8256 r^+y^+ × 8282 r^+y^+	r^+r^+y†	2.5	1.4	1.9	1.0	24.9	26.9	11.0	8.6
(B) 8282 r^+y^+ × 8256.27 $r\ y^+$	$r^+r\ y$†	1.4	1.7	0.7	1.3	40.0	37.6	12.1	15.1
(C) 8282.3 $r\ y^+$ × 8256.27 $r\ y^+$	$r\ r\ y$†	2.6	1.2	2.9	2.7	48.8	45.2	49.8	47.5
Ascospore segregants of diploid (B)‡									
1a $r\ y^+$	$r\ y$	1.0	2.6	1.4	2.8	38.1	34.0	38.2	39.3
1b r^+y^+	r^+y	1.9	1.2	1.1	1.6	37.7	32.1	12.5	13.8
1c r^+y^+	r^+y	1.6	0.9	1.2	1.2	34.2	36.3	15.1	15.9
1d $r\ y^+$	$r\ y$	0.8	1.0	0.5	0.8	36.6	40.2	43.3	39.0
2a $r\ y^+$	$r\ y$	1.7	1.5	1.4	2.0	55.5	48.0	65.0	58.3
2b r^+y^+	r^+y	5.5	4.7	6.6	5.7	34.4	31.5	13.1	11.0
2c r^+y^+	r^+y	1.4	1.6	1.4	2.2	36.2	33.3	10.4	13.3
2d $r\ y^+$	$r\ y$	2.2	1.2	1.3	1.0	45.9	50.0	44.0	46.7
3a $r\ y^+$	$r\ y$	8.2	7.1	6.5	4.4	57.1	64.2	61.0	52.0
3b $r\ y^+$	$r\ y$	5.6	6.0	3.9	5.5	58.7	55.8	64.8	57.7
3c r^+y^+	r^+y	5.5	4.8	4.1	3.4	40.4	44.4	18.1	17.7
3d r^+y^+	r^+y	3.9	3.4	3.7	2.2	42.3	37.7	15.9	14.1
5a r^+y^+	r^+y	2.5	1.2	2.0	1.9	44.8	41.4	26.2	16.6
5b $r\ y^+$	$r\ y$	0.9	1.5	0.4	1.2	49.0	43.9	52.4	55.1
5c $r\ y^+$	$r\ y$	0.8	1.8	0.7	1.5	51.6	47.6	48.8	49.9
5d r^+y^+	r^+y	1.2	1.8	1.1	1.4	39.2	38.8	13.0	11.8
Ascospore segregants of diploid (A)									
1a r^+y^+	r^+y	1.3	..	0.9	..	44.0	...	16.1	...
1b r^+y^+	r^+y	0.4	2.6	0.9	2.8	33.2	37.7	16.6	18.1
1c r^+y^+	r^+y	6.0	..	5.9	..	38.8	...	14.8	...
1d r^+y^+	r^+y	3.9	2.4	4.6	3.0	42.2	36.3	19.2	15.5

* Ultraviolet dose, 1.5×10^3 ergs/mm^2; Dark, without photoreactivation; PR light, after exposure to photoreactivating light for 60 min.
† Inferred.
‡ Genetic constitution of segregants determined by crossing each to respiration deficient tester strains.

rather than gene mutations. We conclude, therefore, that the lack of photoreactivation of the mutation in $r\ y^+$ mutants is expressed as a recessive trait in the heterozygote.

Each of four asci of heterozygote (B) segregated 2 r^+y^+:2 $r\ y^+$ (Table 4), in accord with previous genetic studies on the segregation of normal by genic hybrids (CHEN *et al.* 1950). As shown in Table 3, PR light reduced the ultraviolet-induced frequency of mutants in each of the eight r^+y^+ segregants, but had no effect on the induced mutation frequency in any of the eight $r\ y^+$ segregants. Eight tetrads of a similar heterozygote (8282 $r^+y^+ \times$ 8256.30 $r\ y^+$) yielded results qualitatively similar to those shown in Table 3; namely, that the mutation is photoreactivable in r^+y^+ segregants but nonphotoreactivable in the $r\ y^+$ segregants. Four segregants of a tetrad of homozygous dominant diploid (A) segregated 4 r^+y^+:0 $r\ y$. As shown in Table 3, the mutation induced in each segregant was photoreactivable. The above results show that the photoreactivable *vs.* nonphotoreactivable character of the mutation in question segregates in typical Mendelian fashion and as if under control of the *r* gene pair.

No unequivocal case of back mutation to wild type (r^+y^+) has been observed in mutants carrying the extrachromosomal defect, and repeated attempts by the senior worker to induce such mutations (detectable at a frequency of one per 10^9) by either X-rays or ultraviolet have met with failure. In contrast, 20 of 22 genic stocks thus far studied show a low spontaneous reversion frequency (about one per 10^8) to wild type (PITTMAN 1959). To determine whether the extrachromosomal mutations induced in the back mutant is photoreactivable, a back mutant (8256.27-B r^+y^+) of stock 8256.27 $r\ y^+$ was subjected to 400 sec of ultraviolet and then plated with and without exposure to PR light. The results (Table 4) show that the mutation induced in the back mutant is photoreactivated and to the same degree as that in the normal stock, indicating that the photoreactivable *vs.* nonphotoreactivable character associated with the *r* gene pair is an inherent trait of this gene rather than a closely linked gene.

GIESE and SWANSON (1947) observed that ultraviolet radiation suppressed exogenous respiration and fermentation in yeast. In the present study, restriction of the photoreactivable character of the extrachromosomal damage to respi-

TABLE 4

Photoreactivability of extrachromosomal mutations induced in a respiration-deficient genic (r y^+) *stock and a respiration sufficient back mutant* (r^+y^+) *of this culture*

			Percent mutants Dose					
			Zero		300 sec		400 sec	
Culture			D*	PR light	D	PR light	D	PR light
8256.27 $r\ y^+$	Exp.	1	1.63	1.99	36.6	41.0	71.2	70.3
(genic)		2	2.60	2.71	38.7	38.0	78.9	78.1
8256.27-B r^+y^+		1	0.70	1.12	32.2	14.4	48.1	21.4
(back mutant)		2	1.20	1.03	36.1	15.2	46.2	22.5

* D, plated without photoreactivation; PR, plated after exposure to 60 min of PR light.

ration sufficient stocks suggested that the photoreactivable site is the intact respiratory mechanism. However, as shown in Figure 5, suppression of either exogenous respiration or aerobic fermentation by ultraviolet is nonphotoreactivable, excluding, thereby, this effect as a plausible candidate for the photoreactivable site in question.

DISCUSSION

Interpretation of the genetic control of the above photoreactivation process at the cellular level hinges on what we know of the site and nature of ultraviolet damage producing the extrachromosomal defect. Inhibition of the intact respiratory mechanism falls short as an attractive candidate for the photoreactivable

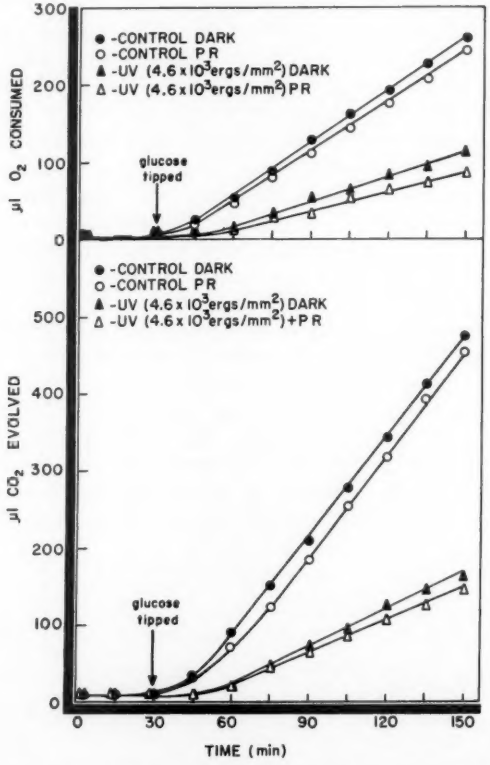

FIGURE 5.—The effect of ultraviolet radiation on respiration (top) and aerobic fermentation (bottom) of normal (r^+y^+) cells with and without treatment with photoreactivating light. Cells were irradiated for 4.6×10^3 ergs/mm^2 and equivalent aliquots (a) held in the dark for 60 min and (b) exposed to PR light for 60 min. Final substrate concentration, one percent glucose; abscissa, incubation time in Warburg reaction vessel.

site(s), since this effect was nonphotoreactivable. The ultraviolet action-spectrum curve for induction of the extrachromosomal mutants (RAUT and SIMPSON 1955) suggests that nucleic acids are some way involved in the mutational process, but whether the photoreactivable site is nuclear or cytoplasmic remains obscure. It would appear that the r^+ allele is not the photoreactivable site, since $r^+\gamma$ rather than $r\,\gamma^+$ (or $r\,\gamma$) mutants were obtained from irradiated normal cells plated without photoreactivation. Disruption, directly or indirectly, of the normal replication (or synthesis) of the extrachromosomal determinants has been postulated as the ultraviolet process subject to photoreactivation (PITTMAN *et al.* 1959). On this assumption, the following plausible hypothesis is formulated to account for the present results: Ultraviolet radiation effects (directly or indirectly) loss of the extrachromosomal factor, that damage being potentially photoreactivable. The *r* gene pair controls the synthesis of a cellular component, either a chromophore or one of several components (enzymes) necessary in the transfer of PR energy to the photoreactivable site. In the presence of the r^+ allele, the energy absorbed by the chromophore is transferred by a sequence of enzymatically controlled reactions to the photoreactivable site, which is subsequently "repaired." In the presence of the *r* allele, PR energy transfer fails to occur since the chromophore (or enzyme) is lacking, and the lesion is expressed at a rate comparable to that in cells plated in the dark. Back mutation of the *r* allele results in the restoration of the ability of the cell to synthesize the component necessary for PR energy transfer. Implicit in this hypothesis is the assumption that there are several steps in PR energy transfer, each under genetic control and, therefore, each presenting a potential block in the photoreactivation process. Indeed, a study of 61 ultraviolet derived genic mutants has shown that at least two genetic blocks occur to prevent photoreactivation of the extrachromosomal mutation, each compensated for in the heterozygote to restore the photoreactivable character of the mutational damage.

The relation between photoreactivation of the extrachromosomal mutation and the inherent ability of the cell to respire suggests that the cellular component necessary for photoreactivation is associated with the aerobic mechanism. This possibility is further strengthened by the fact that the extrachromosomal mutation is photoreactivable in aerobically but not anaerobically grown cells (SARACHEK 1958). Since respiration in yeast is an adaptive process (SLONIMSKI 1953), the postulated component may be adaptively synthesized in aerobically grown, normal cells but absent either in anaerobically grown, normal cells or in mutants carrying the recessive allele of the gene controlling respiration.

An obvious attack on the above hypothesis is to determine the effect, if any, of extracts of respiration sufficient stocks on photoreactivation of extrachromosomal mutations in the genic mutant. Preliminary experiments with 10,000 × G supernatants of normal cell extracts have yielded promising results.

Although our experiments raise the possibility that photoreactivation mechanisms in general are under genetic control, we have shown it for a specific case only. As clearly shown here, photoreactivation of killing is not under direct control of the *r* gene pair. However, along this line, it is interesting to note that

several years ago DR. A. KELNER failed to show photoreactivation of killing in certain yeast strains obtained from DR. C. C. LINDEGREN. While these strains probably were not respiration deficient, it is possible they were genetically non-photoreactivable (DR. A. KELNER, personal communication).

SUMMARY

Extrachromosomal mutations in yeast affecting respiratory ability are induced by ultraviolet radiation in haploid stocks carrying either the dominant or recessive allele of a gene (r) controlling respiratory competence. Photoreactivation of the mutation depends on the allelic state of the gene: In stocks carrying the dominant allele r^+, the mutation is photoreactivable; in stocks carrying the recessive allele r, the mutation is nonphotoreactivable. The photoreactivable *vs.* non-photoreactivable character of the mutation in question segregates in typical Mendelian fashion and as if controlled by the r gene pair. The photoreactivable trait is expressed as a dominant character in the heterozygote.

ACKNOWLEDGMENTS

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THE GENETICS OF A PATTERN

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THIS paper is concerned with the problem of the genetic control of morphogenesis. Previous attacks on this problem have usually taken, as their starting point, some single mutant gene with well-marked morphological effects, and an attempt has been made to detect the earliest effect of this gene. A second approach has been through the study of mosaics (STERN 1954, 1956). The techniques used in this paper are mainly those of quantitative genetics; an attempt has been made to deduce the nature of the developmental processes and their genetic control by studying the range of variation in the end products of these processes, that is, by studying adult variation. It is thus an extension of the line of thought which has led WADDINGTON (1942) to suggest processes of epigenetic canalization. Although a major mutant gene, ocelliless, has been used in our experiments, this gene has been not so much an object of study in itself as a source of observable variation: an environmental shock during development causing comparable variation would have served equally well.

The pattern chosen for study (Figure 1) was that of the three ocelli and the three pairs of associated bristles: the ocellar setae, anterior vertical setae and postvertical setae, lying in the anterior, middle and posterior region of the head respectively. The arrangement of macrochaetae and ocelli shown in the figure

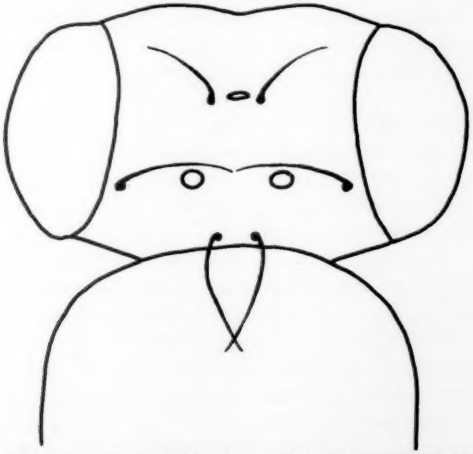

FIGURE 1.—The wild-type pattern of the three ocelli and associated bristles in *Drosophila subobscura*.

is common to all the species of Drosophila we have examined, although various mutants are known which modify it. In *D. subobscura* the sex-linked recessive gene ocelliless removes most of the nine structures shown in most individuals. In the original outbred mutant stock, nearly half the individuals lacked all nine structures, and only one individual out of over 800 was found with all of them. In the remaining individuals, one or more of the ocelli or bristles were present, approximately in their normal positions, the remaining structures being absent. Starting from this population, SONDHI (1960) was able, by breeding from individuals with the greatest number of structures, to obtain a population which, although still carrying the mutant, contained a high proportion of wild-type individuals. In the present experiment, an attempt has been made to produce by selection populations in which a high proportion of individuals have an intermediate pattern with some but not all of the structures present. Two such experiments have been performed, one by breeding from individuals with the two posterior ocelli but lacking the anterior one, and the other by breeding from individuals with the anterior and the left posterior ocelli, but lacking the right posterior ocellus. From the response of the original population to these two types of selection, it has been possible to suggest a hypothesis concerning the genetic control of the wild-type pattern.

EXPERIMENTAL PROCEDURE

The foundation population: As a starting point, a population with a fair proportion of individuals with two ocelli was required. This was obtained by crossing several of SONDHI's (1960) selected lines (the U × U line, which had been selected for the presence of the maximum number of structures, and the two reciprocally selecetd U × D and D × U lines). Seven hundred and seventy-three flies from this foundation population were classified, the presence or absence of each structure being recorded for each individual. Not uncommonly the anterior vertical bristles were repeated, on one or both sides; these repetitions were also recorded.

The main characteristics of the foundation population are shown in Figure 2, and also the characteristics of the U × U line. In these diagrams, the height of the surface at any point is proportional to the frequency in the population of individuals with the corresponding number of bristles and ocelli. (The relationship of these diagrams to WADDINGTON's "epigenetic landscapes" (1957) is as follows; they are three-dimensional cross sections at the end of development of a four-dimensional landscape. They have also been inverted, so that our peaks correspond to WADDINGTON's canals.) In the U × U population, there is a well-marked frequency peak at the wild-type phenotype of six bristles and three ocelli, and an appreciable number of flies with seven or eight bristles, due to the repetition of the vertical bristles. In the foundation population, the frequency of ocelli and bristles is lower than in the U × U line, but still much higher than in the original mutant stock. There is a smaller, but still definite, peak at the wild-type phenotype.

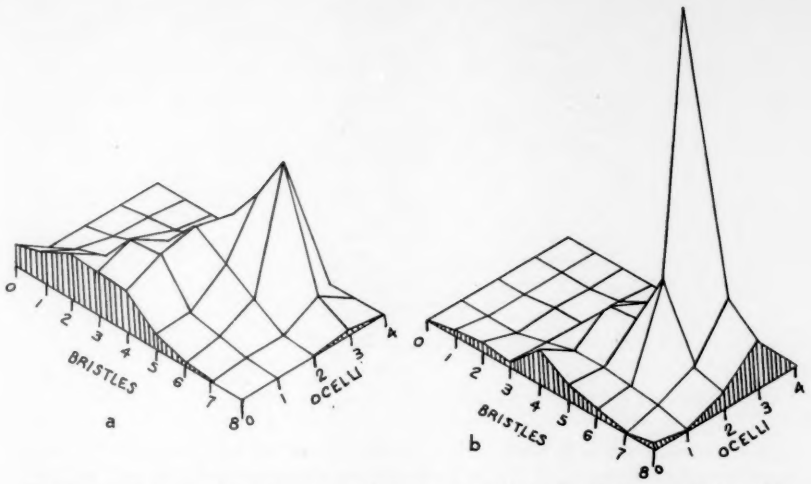

FIGURE 2.—Frequency distributions of phenotypes in (a) the foundation population; (b) a line selected for the wild-type phenotype, SONDHI (1960).

There was a correlation between the presence of bristles and ocelli, as indicated by the ridge running from the positions two bristles, zero ocelli to six bristles, three ocelli. SONDHI (1960) found this correlation to be of general occurrence, so that the same factors, genetic or environmental, which increase the number of bristles, tend also to increase the number of ocelli. This correlation is local as well as general, in that if only some of the ocelli are present, the bristles close to these ocelli are more likely to be present than are the more distantly situated bristles. This point is illustrated in Figure 3.

In ocelliless populations the bristles and ocelli, if present, may be slightly displaced from their normal positions. But in the populations described in this paper the various structures were never displaced to such an extent as to make their identification impossible. There are also variations in the size of the ocelli and, to a less extent, of bristles. These variations have been ignored, but an attempt has been made to be consistent in the scoring of presence and absence.

Selection procedure: Two selected lines, SYM (symmetrical) and ASY (asymmetrical) were maintained.

In the SYM line, only flies with the two posterior ocelli, but lacking the anterior ocellus, were used as parents. Bristles were ignored in the selection of parents. Ten to 12 cultures, with three parents of each sex, were set up in each generation; matings between flies from the same culture were avoided. Selection was continued for 13 generations. Approximately 400 flies (20 males and 20 females from each culture) were classified in each generation. Generations 8, 9, 11 and 12 were not classified, although selection was practiced on them.

In the ASY line, only flies with the anterior and the left posterior ocelli, but

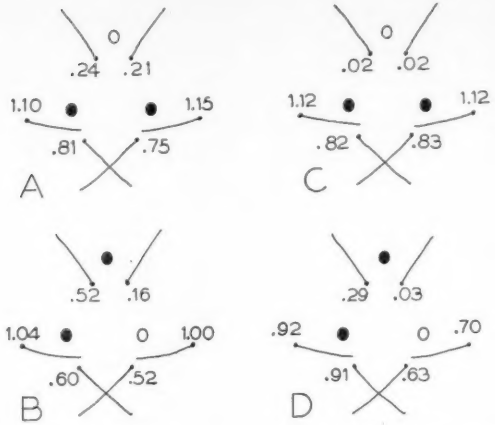

FIGURE 3.—Frequencies of bristles in (A) 114 flies in the foundation population having the two posterior ocelli only; (B) 25 flies in the foundation population having the anterior and left posterior ocelli only; (C) 268 flies in the SYM S–13 having the two posterior ocelli only; (D) 70 flies in the ASY S–12 having the anterior and left posterior ocelli only. The frequency is equal to the number of bristles divided by the number of flies; since the vertical setae are sometimes duplicated, their frequency can be greater than unity.

lacking the right posterior ocellus, were used as parents. Bristles were ignored in selecting parents in the early generations since only a few flies with the required phenotype were available, but in later generations preference was given to flies with more bristles on the left than the right side. Ten to 12 paired matings were set up in each generation, avoiding brother-sister matings. Selection was continued for 12 generations, the number of flies classified varying from 274 to 902.

RESULTS

The response of the two lines to selection is shown in Figure 4. The combined frequency of flies with the anterior and right and with the anterior and left ocelli only has been plotted, instead of the latter frequency alone, since, as will be shown, there was no continued trend towards handedness, so that the combined frequency gives a more accurate estimate of the changes occurring.

In the SYM line the frequency of the selected phenotype increased from 14.75 percent to 64.20 percent, while the combined frequency of the asymmetrical phenotypes declined almost to zero. The frequency distribution of the various phenotypes is shown in Figure 5a. Although selection was practiced only on the ocelli, it had the effect of decreasing the frequency not only of the anterior ocellus, but also of the anterior pair of bristles (the ocellar bristles) while increasing the frequency of the posterior ocelli and of the two posterior pairs of bristles. Hence, there is a striking peak at four bristles and two ocelli, indicating

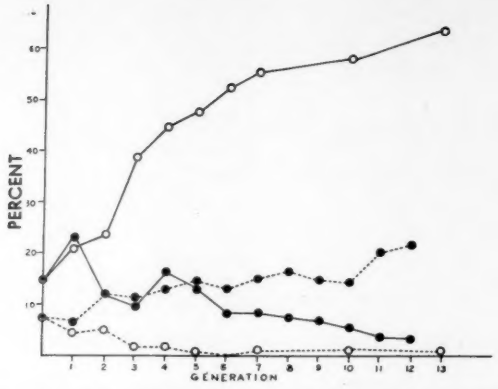

FIGURE 4.—Frequencies of phenotypes in the selected lines. Full lines, frequency of flies with the two posterior ocelli only; broken lines, combined frequency of flies with the anterior and left and with the anterior and right posterior ocelli only; o, SYM line, ●, ASY line.

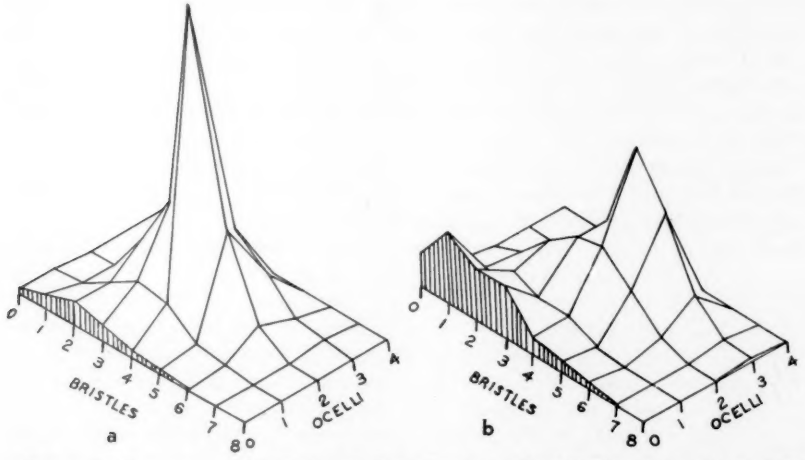

FIGURE 5.—Frequency of distribution of phenotypes in (a) the SYM line, S–13; (b) the ASY line, S–12.

the "canalization" of a phenotype other than the wild type. There was a tendency for the rate of response to decline in later generations, but this merely reflects the reduced intensity of selection in these generations when about half the flies had the desired phenotype.

Response in the ASY line was less striking, although the combined frequency of the two asymmetrical phenotypes increased from 7.24 percent to 21.8 percent. The frequency distribution in generation 12 is shown in Figure 5b. There is no

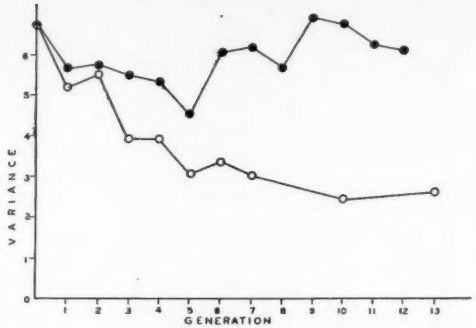

FIGURE 6.—Variance of "score" in o, SYM line, ●, ASY line.

single clear frequency peak. For reasons given below, the frequency of individuals with only one ocellus is low so that there is a valley separating two small peaks at one bristle, zero ocelli and at four bristles, three ocelli.

This contrast between the canalization of a single phenotype in the SYM line, and the absence of such canalization in the ASY line, can be shown in another way (Figure 6). Each individual has been given a "score" equal to the sum of the numbers of ocelli and bristles present and the variance of this score calculated for each generation of each line. There was no significant decline in the variance of the ASY line, whereas in the SYM line, the variance fell to less than half its initial value.

Although the ASY line remained variable, there was a threefold increase in the frequency of the selected phenotype. The nature of the changes in the population responsible for this increase can be analyzed as follows:

let F_{AL}, F_{AR} = frequencies, respectively, of individuals with the anterior and left, and with the anterior and right ocelli only.

F_1 = frequency of individuals with only one posterior ocellus.

$F_{L.1}, F_{R.1}$ = proportions of individuals having only one posterior ocellus which have, respectively, the left and the right ocellus; $F_{L.1} + F_{R.1} = 1$.

$F_{A.L}, F_{A.R}$ = proportion of individuals having, respectively, the left and the right ocellus, which have also the anterior ocellus.

$F_{A.1}$ = proportion of individuals having only one posterior ocellus, which have also the anterior ocellus. (In all populations studied, $F_{A.1} \simeq F_{A.L} \simeq F_{A.R}$).

F_L, F_R = frequencies, respectively, of individuals having the left and the right ocellus, independently of the presence of other ocelli.

$F_{A.LR}$ = proportion of individuals having both posterior ocelli, which have also the anterior ocellus.

r = correlation between the presence of the right and of the left ocellus.

Then the frequency of individuals with the selected phenotype is:

$$F_{AL} = F_1 \times F_{L.1} \times F_{A.L}$$
$$\simeq F_1 \times F_{L.1} \times F_{A.1}.$$

Hence an increase in the frequency of the selected phenotype, F_{AL}, can result from an increase in F_1 (an increase in asymmetry), or in $F_{L.1}$ (an increase in left-handedness), or in $F_{A.1}$ (a symmetrical change, increasing the frequency of the anterior ocellus).

Table 1 shows some values of these frequencies. It shows that the increase in the frequency of the selected phenotype in the ASY line was almost entirely due to an increase in $F_{A.1}$. There was a corresponding decrease in $F_{A.1}$ in the SYM line. Similarly, $F_{A.LR}$ increased in the ASY line and decreased in the SYM line. In other words, the main change in the ASY line was an increase in the frequency of individuals with the anterior ocellus—the opposite change to that which occurred in the SYM line. Consequently, the frequency of individuals with only one posterior ocellus and no anterior ocellus, $F_1(1 - F_{A.1})$, declined in the ASY line, so that, as shown in Figure 5b, there are few flies with only one ocellus.

But the table suggests that changes in handedness and in asymmetry may also have occurred in the ASY line: these possibilities will be considered in turn.

Handedness: Figure 7 shows the changes in $F_{L.1}$, the frequency of left-handed individuals as a fraction of all handed individuals, in the ASY line. For the first seven generations, there was an increase in the proportion of left-handed individuals, but this tendency was not maintained. In the eighth, ninth and tenth generations individuals with the anterior and right ocelli only were selected from the line and mated together: the values of $F_{L.1}$ in their offspring are shown in the figure. In the 11th generation, matings were made of females with front and right ocelli to males with front and left ocelli, and reciprocally; values of $F_{L.1}$ for the offspring of these crosses are also shown. It will be seen that despite the apparent early trend towards left-handedness, later experiments failed to demonstrate the presence of consistent genetic or maternal effects on handedness. The explanation of these results is not clear. It seems likely that the handedness

TABLE 1

Frequencies of phenotypes following selection

	Number of flies	Correlation between posterior ocelli	Frequencies				
	n	r	F_1	$F_{L.1}$	$F_{A.1}$	$F_{A.LR}$	$F_P = \frac{1}{2}(F_L + F_R)$
Foundation population	773	0.44	0.255	0.482	0.284	0.715	0.643
S–8	902	0.44	0.282	0.561	0.587	0.805	0.529
ASY S–11	361	0.41	0.282	0.560	0.726	0.918	0.609
S–12	592	0.43	0.280	0.542	0.777	0.915	0.583
S–7	465	0.48	0.137	0.468	0.047	0.277	0.843
SYM S–10	480	0.38	0.104	0.380	0.060	0.327	0.908
S–13	417	0.44	0.137	0.492	0.070	0.185	0.857

For identification of symbols, see text.

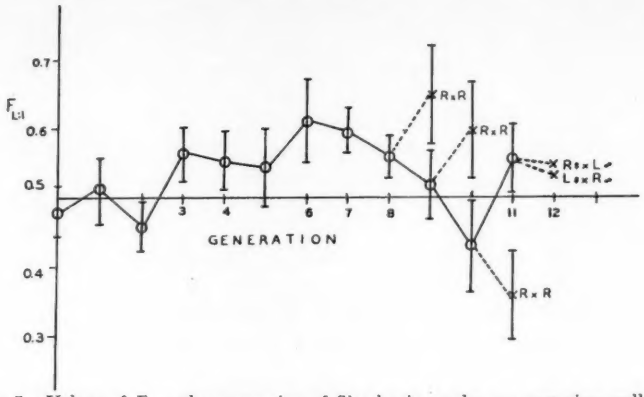

FIGURE 7.—Values of $F_{L,1}$, the proportion of flies having only one posterior ocellus which have the left ocellus, in the ASY line; the vertical lines represent standard errors.

of individuals is a purely chance phenomenon in the sense that it is not influenced either genetically or maternally.

Asymmetry: If there is no significant handedness in the population, then $F_L = F_R = F_P$, say. It is easy to show that F_1, the frequency of asymmetrical animals, is given by $F_1 = 2F_P(1 - F_P)(1 - r)$.

Table 1 shows that r did not change in either line. r measures the relative importance of factors affecting the two posterior ocelli in common to the factors affecting the two ocelli independently.

If r is constant, then F_1 is a maximum when $F_P = 0.5$. For $r = 0.44$, $F_{1max} = 0.28$. The value of F_1 is not very sensitive to changes in F_P from about 0.4 to 0.6; for $F_P = 0.4$ or 0.6, $F_1 = 0.269$.

The table shows that in the foundation population the value of $F_P = 0.643$ was too high to give the maximum value of F_1. In SYM line, F_P rose further, with a corresponding decline in F_1. In the ASY line, F_P fell to between 0.5 and 0.6, and F_1 rose to 0.28 which is the maximum possible without a reduction in r. Thus, although there was a slight increase in the frequency of asymmetrical flies in the ASY line and a large decrease in this frequency in the SYM line, these changes were the consequences of a symmetrical change, that is, of a change in F_P affecting both posterior ocelli equally.

In conclusion, the increase in the frequency of the selected phenotype in the ASY line was the consequence of two symmetrical changes, the first and most important being an increase in the frequency of the anterior ocellus, and the second (and less important) a decline in the frequency of the posterior ocelli nearly to the optimum value of 0.5.

DISCUSSION

The experimental results are most easily interpreted in terms of a hypothesis concerning the genetic control of the development of the pattern. It is supposed that four systems of genes are required, as follows (Figure 8):

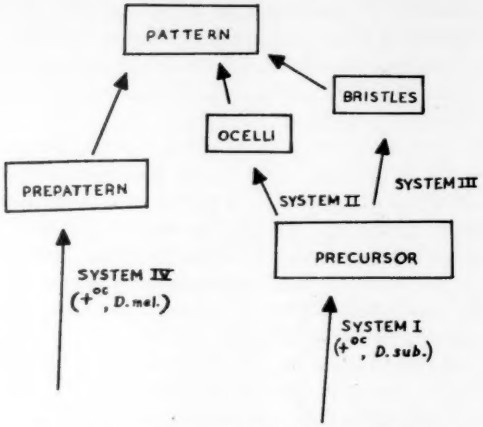

FIGURE 8.—Genetic control of the ocellar pattern.

System I is responsible for the formation of a common "precursor" of both ocelli and bristles; this precursor must be distributed in adequate amounts over the region where the pattern is formed. (It should be emphasized that this precursor is hypothetical; it might be better to talk of the distribution of competence to form bristles and ocelli.)

Systems II and III are responsible for the formation of ocelli and bristles respectively, given that the necessary common precursor is present.

System IV specifies the position at which ocelli and bristles are formed; this corresponds to what STERN (1954, 1956) has called a "prepattern."

Both ocelli and bristles are derivatives of the hypodermis, so it is reasonable to postulate a common precursor. BIRMINGHAM (1942) has shown that cephalic imaginal discs, implanted in the body cavity, commonly develop the bristles and ocelli appropriate to the side of the head from which the implant was taken, including a small median ocellus, probably corresponding to half the normal anterior ocellus. It follows that the symmetrical pattern of adult flies arises from the bringing together of two asymmetrical half-patterns, and that the capacity to form a "prepattern" is a property of the imaginal discs themselves.

The $+^{oc}$ gene in *D. subobscura* (i.e., the wild-type allele of ocelliless), is part of system I. In the original mutant population, the amount of precursor was small, and its distribution irregular. But systems II, III and IV were present in this population since some individuals did form ocelli and bristles which, if formed at all, were in their normal positions. Which particular structures were formed in any individual depended on the distribution of the precursor. In contrast, flies homozygous for the ocelliless mutant in *D. melanogaster* have no ocelli, but have a number of irregularly arranged bristles on the top of the head. It seems, therefore, that the wild-type allele of this *melanogaster* mutant forms part of system IV responsible for the prepattern.

This account oversimplifies the situation in one respect: the ocelliless mutant in *D. subobscura* does have some effect on the prepattern. The bristles, and much more rarely the ocelli, may be duplicated. Often the structures are slightly displaced, and very occasionally an additional bristle occurs in an atypical site. But the present selection experiments have altered the distribution of the precursor rather than the prepattern. This conclusion is drawn because in the SYM line, in those flies which did have three ocelli, the anterior ocellus was usually reduced in size, often to a small speck, but was not displaced posteriorly.

SONDHI's (1960) experiments showed that selection in ocelliless populations could alter the amount of precursor in either direction. In other words, it was possible to select a polygenic system capable in large measure of performing the function normally performed by the $+^{oc}$ allele. The present experiments show that it is also possible to alter the distribution of the precursor along the antero-posterior axis. In the SYM line, the precursor was concentrated at the back of the head, whereas in the ASY line the amount of precursor posteriorly was slightly reduced to the level which gave a probability of approximately 0.5 for the development of a given posterior ocellus and, hence, the maximum chance for the development of an asymmetrical individual.

The failure to alter r, the correlation between the posterior ocelli, or to produce any marked degree of handedness suggests that the distribution of the precursor along a lateral axis was not under genetic control. This is not surprising. The alteration of the antero-posterior distribution of the precursor during development probably depends on the existence earlier in development of antero-posterior gradients in the head region. There are probably no corresponding lateral gradients in the head of developing Drosophila since the adult structure is symmetrical.

If this interpretation is accepted, it follows that the success in reducing the phenotypic variance in the SYM line depended on the effectiveness of *directional* selection in favour of genes concentrating the precursor posteriorly and on the presence of the prepattern; it did not depend on "normalizing" selection (WADDINGTON 1957) in favour of intermediates for a single quantitative character. Such normalizing selection is usually relatively ineffective in altering a population (FALCONER and ROBERTSON 1956; ROBERTSON 1956). To use a homely analogy between the distribution of phenotypes in a population and the distribution of dust on a floor, our experiment demonstrates the effectiveness of brushing in a single direction, corresponding to directional selection, used in combination with a dustpan, corresponding to the existence of a constant prepattern in all members of the population.

Unfortunately, if all morphological constancies in development were to be explained in terms of pre-existing morphological constancies present at an earlier stage of development, this would lead by an infinite regress to a preformationist theory of embryology. No light has been shed by our experiments on the genesis of the prepattern itself. The theoretical problems of the genesis of such constant prepatterns have been discussed by MAYNARD SMITH (1960), taking as a starting

point the analysis by TURING (1952) of the origin of chemical instabilities in an initially homogeneous field. Experimentally, it is hoped that something may be learned about the genesis of the ocellar pattern by selection which concentrates on the displacements of the ocelli and bristles, rather than on their presence or absence.

SUMMARY

The mutant ocelliless in *Drosophila subobscura* affects the three ocelli and three pairs of bristles on the top of the head. In most flies homozygous for the mutant, one or more of these nine structures are absent, but if present, the structures are approximately in their normal positions.

This paper describes the changes in two selected lines homozygous for the mutant. In the SYM (symmetrical) line, flies with the two posterior ocelli only were selected as parents; in the ASY (asymmetrical) line, flies with the anterior and left posterior ocelli only were selected as parents.

The results can be explained if it is assumed that the wild-type pattern depends on two genetic systems, the first responsible for a "prepattern" determining the positions of the ocelli and bristles, and the second for the formation of a common "precursor" of ocelli and bristles. The wild-type allele of *oc* forms part of the second system, so that in ocelliless flies the precursor is abnormal in amount and distribution. Selection can alter the amount of precursor, and its distribution along an antero-posterior gradient, but cannot modify its distribution along a gradient from left to right.

Consequently the SYM line contained a high proportion of individuals with the two posterior ocelli and the two posterior pairs of bristles only, due to a concentration of the precursor posteriorly. But in the ASY line, selection did not alter either the correlation between the presence of the left and right ocelli, or the relative frequency of flies with the left as compared with the right ocellus only. Therefore, although there was a threefold increase in the frequency of the selected phenotype in the ASY line, this was the result of symmetrical changes in the distribution of the precursor.

ACKNOWLEDGMENT

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INHERITED VARIATIONS IN THE ESTERASES OF TETRAHYMENA[1]

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THE technique of zone electrophoresis in starch gels was developed by SMITHIES (1955) and used in genetic studies of the serum proteins of man and cattle (SMITHIES and WALKER 1955; SMITHIES and HICKMAN 1958). To this technique HUNTER and MARKERT (1957) coupled certain histochemical procedures for identifying the enzymatic properties of electrophoretically resolved proteins from tissue homogenates. The combined electrophoretic and histochemical techniques have recently been applied to an analysis of the esterases of variety 1 of the Protozoan, *Tetrahymena pyriformis* (ALLEN 1959). One class of these esterases is eserine sensitive and activated by sodium taurocholate. Since this class shows strain differences, a genetic analysis of these differences was initiated. The present report is concerned with a description of these esterases and the manner in which differences among them are inherited within the clone and in breeding experiments. The results of this analysis show that a complex system is involved and that the system is under genetic as well as epigenetic (NANNEY 1958) control.

MATERIALS AND METHODS

Material: Variety 1 of *T. pyriformis* contains four inbred strains (families), A, B, C, and D, each of which was derived from a wild isolate by selection and inbreeding for various mating type alleles (NANNEY 1959b). Strains A and B were derived from a cross of wild strains WH–6 and WH–14; each has been inbred for at least eight generations. The origins of strains C and D are not clear. Strain C was supposedly derived from a cross of wild strain Alp–4 and strain B and then inbred for five generations. Strain D was supposedly derived from a cross of wild strain UM–226 and strain B and inbred for seven generations. However, the esterase patterns and the distribution of serotypes (ALLEN, unpublished; LOEFER, unpublished) show similarities between strains C and UM–226 and similarities between strains D and Alp–4. This observation suggests that strain C probably arose from UM–226 and strain D from Alp–4. A resolution of this problem awaits further investigation. In the work to be reported below crosses were performed between strains using the most inbred member of each strain. Most of the work concerns strains B and C and their hybrids.

Maintenance of clones: Each clone was maintained on bacterized medium and on a sterile peptone medium. The bacterized medium was prepared by inoculating Cerophyl (rye grass) infusions with *Aerobacter aerogenes*. Tubed cultures

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of each clone were maintained in the bacterized medium at 16°C; these served as the source of the parents in the earlier crosses. The sterile medium contained one percent proteose-peptone broth adjusted to *p*H 7.0. Tubed cultures of each clone were stored in the peptone medium at 16°C; these served as the source of flask cultures, which were grown at 30°C and used in making extracts for the esterase analysis.

Crosses: The most recent crosses have been performed under sterile conditions using washed animals grown in one percent proteose-peptone. All isolations and transfers were made into peptone medium. The earlier crosses were carried out in bacterized medium. In either case the crosses were performed at 30°C and the general procedures of NANNEY and CAUGHEY (1955) were followed. Non-conjugants were eliminated and the exconjugants were carried to maturity by serial transfers of single cells (unless otherwise specified). Mature cells, when grown on the bacterized medium, were then transferred by aseptic technique to the sterile medium by a series of transfers in sterile glass-distilled water and antibiotics (streptomycin and penicillin).

Starch gel electrophoresis: Erlenmeyer flasks (250 ml) containing 100 ml of sterile peptone medium were inoculated with 1 to 5 ml of a tubed plateau culture and grown for five to seven days at 30°C (plateau) unless otherwise specified. Crude extracts of concentrated cells were prepared by repeated freeze-thawing. A filter paper (Whatman #1) of known dimensions was dipped into the crude extract and inserted into a transverse cut in the starch block. Electrophoresis was then carried out in the horizontal position (SMITHIES 1955) for five hours at room temperature using a borate buffer system (0.03 M in the starch; 0.3 M in the end trays) at *p*H 7.0–7.5. An eight-volt drop per centimeter through the starch was generated by a Heathkit Variable Voltage Regulated Power Supply Kit (Model PS-3).

Histochemical techniques: The starch blocks were divided into three equal strips by horizontal cuts and a different histochemical test was made on each strip (HUNTER and MARKERT 1957; MARKERT and HUNTER 1959). The substrates employed were alpha naphthyl acetate, beta naphthyl acetate, alpha naphthyl propionate, and alpha naphthyl butyrate; the stable diazotate of 4′-amino–2′,5′–dimethoxybenzanilide (Fast Blue RR, General Dyestuff Co.) was used as dye coupler. The substrate (0.01 percent) and dye coupler (1 gm/L) were added to 0.1 M phosphate buffer at *p*H 7.4 and the reaction carried out at room temperature (PEARSE 1953). At the sites of enzymatic activity in the starch strips the naphthol molecule is split off and coupled to the diazonium salt to give rise to a colored band. For inhibition studies, eserine sulfate, at a final concentration of 10^{-4} M, was added one hour before the substrate and dye coupler were added to the reaction mixture. Sodium taurocholate, at a final concentration of 10^{-2} M, was added to the reaction mixture at the same time as the substrate and dye coupler. After four hours of incubation, the starch strips were removed from the reaction mixture, washed in distilled water, and photographed using a Polaroid Land camera. The intensity of staining of the various bands, which is roughly proportional to the activity of the enzyme, was determined directly from some of

the starch strips with a modified Photovolt Densitometer (Model 525, modified by DR. ROBERT L. HUNTER in cooperation with the Photovolt Corporation).

Characterization of the esterases

About 20 bands with esterase activity can be separated in the starch gel from extracts of variety 1 of *T. pyriformis*. The esterases can be divided into two classes by their response to 10^{-4} M eserine sulfate (Figure 1). The eserine insensitive class will be treated in more detail elsewhere (ALLEN, unpublished); it includes a motley array of esterases, some with restricted specificities and others with broader specificities. Some of these esterases are activated by 10^{-2} M sodium taurocholate and others are unaffected. Some of the enzymes of this class are weak and appear only in preparations of high protein concentration; hence, their detection tends to be somewhat erratic. With certain exceptions, most of them are found in all of the strains and hybrids. The relationship between esterases of this class and the total pattern of esterases is not clear.

In the present paper attention will be confined to the class of esterases which is sensitive to eserine, and which, by this criterion, should contain the cholinesterases. Nine to ten bands possessing this type of activity migrate as two groups of bands, labelled B and C in Figure 1. The B group contains five bands and migrates toward the cathode at *p*H 7 to 7.5, while the C group contains four or five bands and migrates toward the anode. Figure 2 shows that the B group is found in strains A, B and D, but that the C group is limited to strain C. For further characterization of these groups of bands, strain B was selected as representative of the B group and strain C as representative of the C group. Except for a very few crosses, no further work has been done with strains A and D.

All of the bands of this class respond in a similar manner to inhibitors and activators—behavior which makes these esterases difficult to classify. Thus, as shown in Figure 4, *all* of the bands (B, on the right; C, on the left, of the same starch strips) are inhibited by 10^{-4} M eserine sulfate and *all* are enhanced by 10^{-2} M sodium taurocholate. The inhibition by eserine would result in their being classified as cholinesterases, while the activation by taurocholate would result in their being classified as lipases (GOMORI 1952). The chemical basis of the enhancement of these bands by taurocholate is not known and it may involve changes in the starch matrix itself. Unfortunately, the ability of these enzymes to hydrolyze choline esters cannot be tested directly with these techniques since the available compounds cannot be employed in the coupling reaction. Thus, a classification of these esterases cannot be satisfactorily made at present, and, in order to avoid controversy, they will be designated class I esterases, or eserine sensitive esterases. The eserine insensitive class will be referred to hereafter as the class II esterases.

In addition to their similar patterns of inhibition and activation, all of the class I esterases have similar affinities for the substrates used, as shown in Figure 3. They can split alpha naphthyl acetate, beta naphthyl acetate and alpha naphthyl propionate, but they cannot split alpha naphthyl butyrate. If the class I esterases are, in fact, cholinesterases, these specificities would suggest that their natural

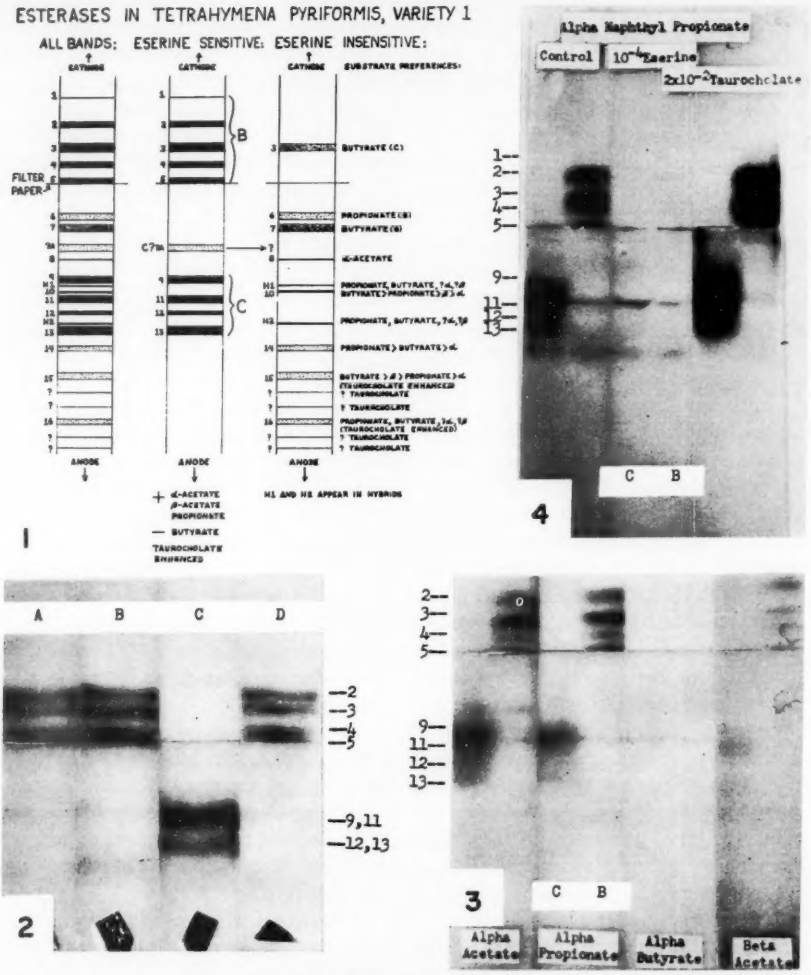


FIGURE 1.—Diagram of esterases in *Tetrahymena pyriformis*, variety 1. FIGURE 2.—Distribution of B and C groups of class I esterases in strains A, B, C and D. FIGURE 3.—Affinities of B and C groups of class I esterases for four substrates: alpha naphthyl acetate, alpha naphthyl propionate, alpha naphthyl butyrate, and beta naphthyl acetate. Two preparations were placed on each starch column—B on the right and C on the left. FIGURE 4.—Effect of eserine sulfate and sodium taurocholate on B (right) and C (left) groups of class I esterases with alpha naphthyl propionate as substrate.

substrate may be acetylcholine, since acetylcholinesterase splits butyrylcholine very poorly (see p. 278. DIXON and WEBB 1958, for a discussion of work by ADAMS and WHITTAKER).

An eserine sensitive esterase, identified by SEAMAN (1951; SEAMAN and HOULIHAN 1951) in a related strain of *T. pyriformis* as an acetylcholinesterase, was found to be associated with the cortex of the cell. The activity of this enzyme was concentrated in a pellicular fraction obtained by cell fractionation procedures. Eserine sulfate, at a concentration of 10^{-4} M, was found to block ciliary movement *in vivo*. SEAMAN (1951) speculated that the fibrillar system in Ciliates acts as a primitive conducting mechanism analogous to nerve fibers.

Cell fractionation procedures have not, as yet, been applied to the present system. However, unsuccessful attempts were made to determine the intracellular distribution of these class I esterases using localization techniques with the above substrates and inhibitors (ALLEN 1958; and unpublished). Although characteristic distributions are obtained, these techniques did not reveal any strain differences nor did they distinguish between the two classes of esterases.

Although no differences in *specificity* could be found between bands having class I esterase activity, differences in the *amount* of activity were observed as a reflection of different growth conditions. A systematic investigation of these quantitative differences was undertaken using two variables: the growth cycle and the conjugation sequence.

Under the conditions employed, the various stages of the growth cycle were reached after the following days of growth: logarithmic phase—zero to two days; plateau—five to seven days; stationary—ten days; decline—14 days. Samples of strains B and C were obtained at (a) 0.5 or one day, (b) two days, (c) five days, (d) seven days, (e) ten days, and (f) 14 days. These samples were adjusted to a similar concentration of cells using equal volumes of material with the same optical density (using a Photovolt Lumetron Colorimeter). A photograph of the results with the two strains (Figures 5 and 6) shows that differences in the amount of activity of these esterases did occur at different stages of the growth cycle. These differences were semiquantitated with the Densitometer. The starch strips were thin and transparent, thus permitting a setting of 100 percent transmission of light (zero percent absorption of light). The stained areas were scanned for their maximum absorption of light. It is these values which make up the "density" curves of Figures 7 and 8. Throughout the growth cycle a characteristic curve may be drawn for each band. In strain B, band 1 changed little, band 3 had a high value during the logarithmic phase of the growth cycle, bands 2 and 5 achieved their highest values during plateau, and band 4 reached its maximum value during the stationary phase. In strain C, two bands (12 and 13) changed little, while two other bands (9 and 11) reached their maximum values during the early part of the growth cycle.

The ordered sequence of activities of the members of each group of esterases during the growth cycle might occur under two different circumstances. The changes could reflect shifts in the composition of the population of cells—and for each esterase there could be a different cell type. Or, the changes could occur in

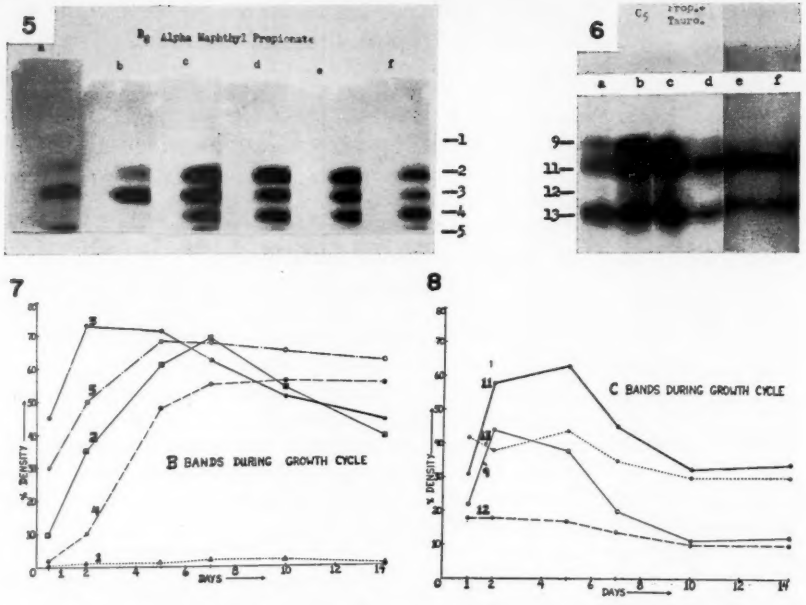


FIGURE 5.—B bands during growth cycle: (a) 0.5 days (b) two days (c) five days (d) seven days (e) ten days (f) 14 days. Substrate: alpha naphthyl propionate. FIGURE 6.—C bands during growth cycle: (a) one day (b) two days (c) five days (d) seven days (e) ten days (f) 14 days. Substrate: alpha naphthyl propionate, with sodium taurocholate. FIGURE 7.—Curves of B bands during growth cycle based on Densitometer readings. Percent density refers to percent absorption of light. Substrate: alpha naphthyl propionate. FIGURE 8.—Curves of C bands during growth cycle based on Densitometer readings. Substrate: alpha naphthyl propionate.

a single cell type and reflect shifts in the internal environment of the cell. The first possibility can probably be discarded since no gross heterogeneity of cell types was suggested in a study of subclones of strain B. Table 1 shows that subclones were alike at each of two stages tested in the growth cycle (two days and five days). Thus, each cell probably produces all of the enzymes of one of the groups of esterases, and, as the cell's internal environment changes during the growth cycle, shifts in activity of these enzymes result. It is possible, of course, that each of these enzymes plays a slightly different biological role in the economy of the cell.

During conjugation a different pattern of variation in activity of these esterases is observed. Using aseptic technique, a large population of cells was collected in the logarithmic phase of the growth cycle and was washed in three changes of glass-distilled water to get rid of the peptone medium. The washed cells were resuspended in distilled water, which was changed after 24 hours. Then, similarly treated cells of a different mating type were added and the mixture was

TABLE 1

Densitometer readings of bands in B subclones at two days and five days during growth cycle

	Two days					Five days				
Band:	1	2	3	4	5	1	2	3	4	5
Subclone										
1	<1	24	24	±1	16	1	25	31	28	20
2	1	26	34	1	19	1	28	32	20	21
3	<1	18	27	1	18	1	20	20	17	16
4	<1	19	27	1	12	1+	35	39	27	32
5	<1	15	26	1	16	1	28	32	23	25
6	<1	19	25	1	10	1+	33	37	32	30
7	<1	13	19	1	13	1	24	35	21	21
8	<1	21	21	1	16	1+	40	48	35	41
Average	<1	19.4	25.4	±1	15.0	1+	29.1	34.2	25.5	25.8

placed in a New Brunswick variable speed shaker overnight. The following day the mixed population was removed from the shaker and dispensed into depression slides where conjugation occurred some 45 minutes later. The procedure of shaking the cells was introduced in the hope of activating the cells for mating so that synchrony in mating might result with the termination of shaking. However, only partial success was achieved: after shaking, more cells mated sooner, but not all of the cells did so. At various times over the next 48 hours samples were collected, pooled, and extracts made of concentrated cells. These extracts were tested by the procedures described above.

Only trends in the data can be pointed out, since the biological aspects of conjugation have not been completely controlled. It was not possible to make all the animals conjugate nor to synchronize them. By the end of the experiment about 80 percent of the population was estimated to have conjugated on the basis of direct cytological examination employing the Feulgen technique.

Some of the observed changes were shown in preliminary tests to be due to starvation, a necessary prerequisite for the elicitation of conjugation. First, the total enzymatic activity of the preparation fell to about 1/32 that of the activity of preparations of feeding cultures. Second, the activity of one of these esterases (C–12), usually the least active in feeding cells, was more active in starved cells (Figure 9: C × C, sample 1).

Other changes, however, were observed during conjugation, which were not observed in starved cultures; these were observed in the intrastrain crosses as well as the interstrain cross (Figures 9 and 10). Certain esterases decreased in activity (B–2; C–9); certain esterases changed little (B–3; C–11); and certain esterases increased in activity (B–4,5) or remained high in activity (C–12,13). Thus, as conjugation proceeded, a shift in activity was observed from the more negatively migrating esterases to the more positively migrating esterases, and the same shift occurred in both the B and C groups of enzymes. This shift in activity might have resulted from a changing intracellular environment accompanying conjugation, and a similar shift in cells of different phenotype might

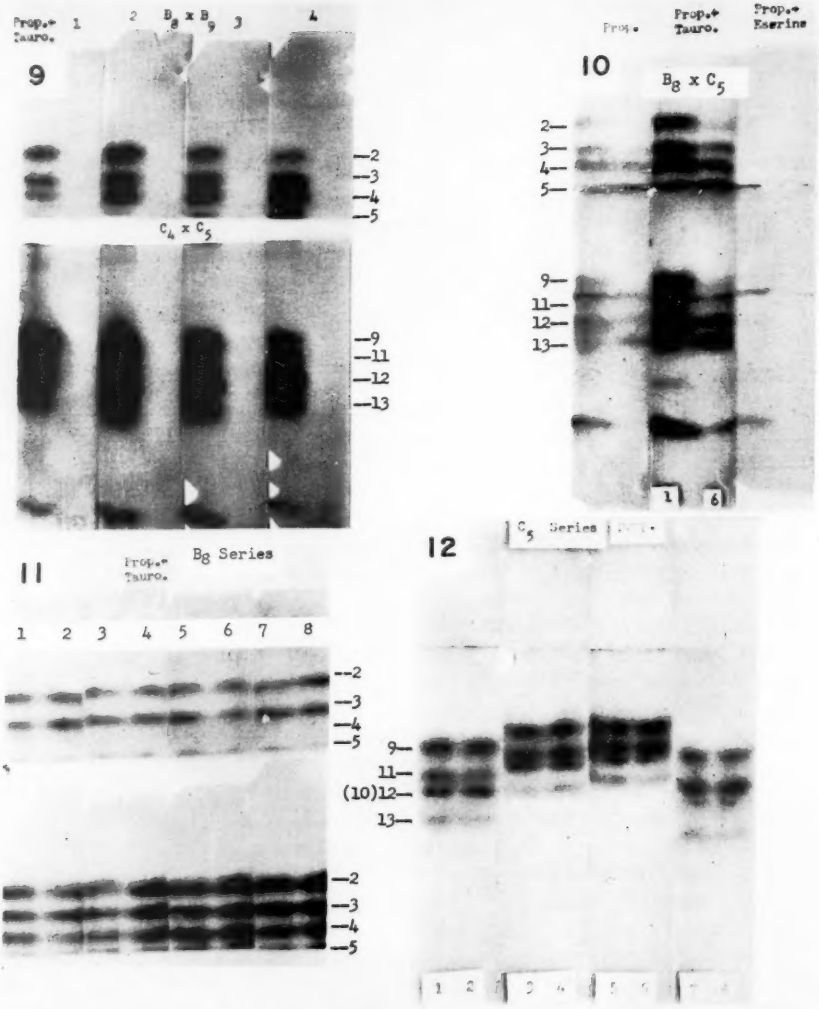


FIGURE 9.—*Top:* B bands during successive stages in conjugation of B × B. *Bottom:* C bands during successive stages in conjugation of C × C. (1) Before conjugation started; (2) 12 hours after conjugation began; (3) 24 hours after; (4) 36 hours after. Substrate: alpha naphthyl propionate, with sodium taurocholate. FIGURE 10.—B and C bands during successive stages in conjugation of B × C. (1) Before conjugation; (6) 48 hours after the beginning of conjugation. Substrate: alpha naphthyl propionate—alone, with sodium taurocholate, and with eserine sulfate. FIGURE 11.—Eight B subclones sampled at two days (top) and at five days (bottom). Substrate: alpha naphthyl propionate with sodium taurocholate. FIGURE 12.—Eight C subclones sampled at five days. Substrate: alpha naphthyl propionate.

reflect similar environmental changes. On the other hand, if each enzyme served a slightly different biological function in the metabolism of the cell, this might argue for enzymes with homologous function in the two groups of esterases.

A systematic investigation of the variations in activity of the class I esterases has shown that a sequence of changes occurs during the growth cycle and during conjugation. These variations were not random fluctuations in amount of enzymatic activity but were ordered and depended upon specific growth conditions. In general, these observations are similar to those of MARKERT and MØLLER (1959) on the lactate dehydrogenases of the pig and other species. They refer to the electrophoretically distinct forms of an enzyme as *isozymes*. Using eight different substrates, they could detect no differences in specificity between these isozymes. Yet, they could show characteristic variations in the amount of enzymatic activity of each isozyme from several different tissues and in the embryonic stages of the same tissue. Their data did not permit genetic interpretations, but they suggested that each isozyme might be controlled by a separate gene or, alternatively, that all the isozymes might represent modifications of a single gene product. As will become apparent in a succeeding section of this paper, a definitive answer to this question has not, as yet, been obtained in Tetrahymena, but the results to date are more compatible with their second hypothesis.

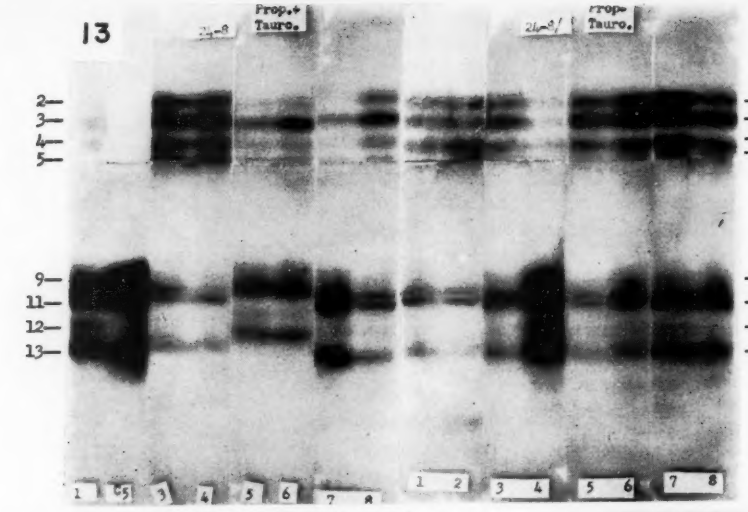
Inheritance during clonal multiplication

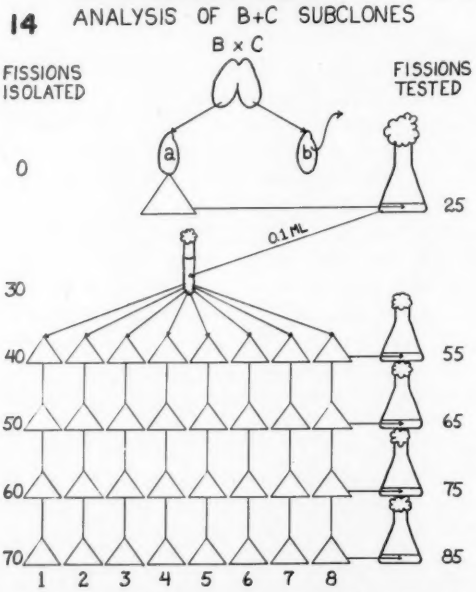
As a clone is propagated by successive fissions, three patterns of inheritance of the class I esterases can be demonstrated. Two of these patterns are stable and show only the quantitative variability associated with growth cycle changes. These are the B and C patterns, as exemplified by strains B and C. Either the B or the C group was perpetuated in the clone since subclones of strains B and C showed only the isozymes characteristic of each strain (Figures 11, 12).

The third pattern of inheritance, on the other hand, exhibited clonal variability and was found in certain hybrids between strains B and C. All of the B isozymes and all of the C isozymes were present in these clones, but variations in the amount of activity of the one or the other *group* of isozymes was observed in different subclones. This variability is illustrated in Figure 13 by two sets of eight subclones. It is apparent that some of the subclones have more B-group than C-group activity, others have more C-group than B-group activity, and others are equally active for both groups of isozymes.

The perpetuation of the pattern of each B+C subclone was followed over the course of 40 fissions by the technique of serial transfer of single cells at 10-fission intervals. The "leftover" populations were inoculated into flasks to provide large populations for making extracts. The design of this experiment is illustrated in Figure 14.

The results of this experiment showed: first, that each pattern is perpetuated rather closely in successive fissions; and second, that changes in pattern do occur, but that the rate of change is slow. Quantitative data on all the subclones appear in tabular form (Table 2), based on Densitometer readings. Here, in order to equate the total activities of different samples, the activities of the B group iso-





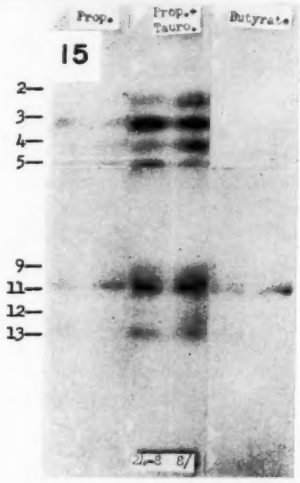


FIGURE 13.—Two sets of eight B+C subclones, sampled at five days—one set from 24–8, the other from 24–8/. Substrate: alpha naphthyl propionate, with sodium taurocholate. Note that 24–8–2 died and was replaced by a C clone. FIGURE 14.—Design of experiment testing B+C subclones as a function of fission. FIGURE 15.—B+C clones (a) 24–8 and (b) 24–8/; exconjugants from the same pair, derived from a cross of B × C. Substrates: alpha naphthyl propionate—alone, with sodium taurocholate; and alpha naphthyl butyrate.

TABLE 2

Percentage of C-group activity in B + C subclones at ten fission intervals

Subclone:	1	2	3	4	5	6	7	8	Mean
No. fissions after conjugation	Exconjugant a (24–8)								
55	74	..	24	18	63	53	66	43	49
65	..	..	28	25	..	72	..	36	40
75	100	..	25	26 22	..	67	..	25	44
85	100	42	11	22 16	..	60 86	..	27	46
	Exconjugant b (24–8/)								
55	25	16	35	87	26	36	33	36	37
65	20	14	41	74	22	34	38	58	38
75	23	12	49	61	22	43	39	56	38
85	20	13	31	60	22	45	37	52	35

zymes and the C group isozymes were totaled and the percentage of C-group activity calculated. Thus, 100 percent signifies absence of detectable B-group activity, and zero percent signifies absence of detectable C-group activity. The percentage of C-group activity is presented for each sampling of each subclone. Although the activities of different subclones are markedly dissimilar (compare 1b *vs.* 2b *vs.* 3b *vs.* 4b, etc.), successive samples of any one particular subclone have very similar activities (compare successive samples of 1b, 2b, or 7b). Yet, shifts in the C direction occur in subclones 6a and 8b, and shifts in the B direction occur in subclones 8a and 4b. Nevertheless, in spite of the initial variability, the rate of change with fissions apparently is not very great, and the mean activity of each clone changes very little. This suggests that small differences in the groups of esterases are maintained in different cell lineages. Whether segregation of cells phenotypically "pure" for B or pure for C could occur with additional fissions was not determined, although in the last sampling of one of the subclones (1a) only C isozymes were present.

This study suggested that all three patterns of inheritance of class I esterases—B, C and B+C—can be maintained in different cell lineages for many cell generations. The first two patterns, which are found in different strains, seem to be entirely stable, while the third pattern, which is found in certain hybrids, is "metastable"—and persists with small changes at each cell division. Therefore, each of these types of clones can be used in breeding experiments with a fair assurance of reproducibility of the results.

Breeding behavior

In order to determine how the class I esterases are inherited during sexual reproduction, a series of crosses was made within strains and between strains. Within a strain each group of esterases bred true; thus, in a cross of B × B, only the B isozymes appeared in the offspring, and in a cross of C × C only the C iso-

zymes appeared in the offspring. Crosses between the two strains indicated that the class I esterases are under genetic control but that the control is modified in some manner so that it appears complex. The results of the initial crosses are summarized in Table 3.

When strains B and C were crossed, only the B isozymes appeared in the progeny (for the present the exceptional clone obtained with a repetition of this cross will be ignored). This was also the result obtained from crosses of other strain combinations (A × C, C × D, A × B, A × D, B × D). Exconjugants from the same pair were found to have identical phenotypes, i.e., both had B group isozymes. This result would be expected if the trait were controlled by nuclear genes and B were dominant to C, since the exconjugants from the same pair are alike genetically. However, it would also follow if a cytoplasmic element were involved and if B cytoplasm "dominated" C cytoplasm in a mixture of cytoplasms brought about by cytoplasmic exchange.

To test for a simple genetic basis, a backcross of the F_1 (B × C) hybrid to the C parent was made. If the trait were inherited as a difference at one or two loci, simple 1:1 or 3:1 ratios would be expected. Instead, 19 out of the 20 progeny were B in phenotype, and the remaining one had all of the B and all of the C isozymes. Here exconjugants from the same pair were identical in phenotype—including the exceptional pair. Not only were simple genetic ratios not obtained in the backcross of (B × C) × C, but this result was also observed in an F_2 from a cross of D × C; all 18 of the progeny had B isozymes only.

Such behavior might be attributed to the participation of polygenes in the determination of this character. Hence, a second backcross of two "B" hybrids

TABLE 3

Distribution of class I esterases among the progeny of inbred strains and their hybrids

Phenotype	Cross	No. pairs tested	No. pairs with:†		
			B group	C group	B+C groups
B,B	B × B	10	10	0	0
C,C	C × C	22*	0	22	0
B,C	B × C	10*	10	0	0
B,C	B × C	16‡	15	0	1
B,C	A × C	5	5	0	0
B,C	D × C	5	5	0	0
B,B	A × B	5	5	0	0
B,B	A × D	5	5	0	0
B,B	B × D	5	5	0	0
B,B	(D × C)F_2	18	18	0	0
B,C	(B × C) × C	20	19	0	1
B,C	(B × C)C × C	26	25	0	1
B,C	(B × C)C × C	24*	23	0	1

* Two cells selected at random from pair cultures rather than the usual procedure of separating exconjugants.
† Wherever tested, the two exconjugants from the same pair were always identical in phenotype.
‡ Cross performed in peptone medium; exconjugant cultures tested at 25 fissions. All other crosses in this table performed in bacterized medium and exconjugant cultures tested at about 100 fissions after conjugation.

(B × C) C to the C parent was made. If the simultaneous presence of C alleles at several loci were necessary in order to produce the C phenotype, some C-group offspring would be expected to appear in a sample of about 25. This expectation was not realized. Instead, the results were similar to the first backcross generation: most of the offspring were B in phenotype, but in each cross, one clone had all of the B isozymes and all of the C isozymes. Where tested, the exconjugants from the same pair were alike in phenotype. This, of course, does not eliminate the possibility that a large number of polygenes might be involved, but it suggested that a repetition of the original cross (B × C) might be more instructive.

A repetition of this cross was suggested on other grounds. Studies of the B × C cross during the course of conjugation indicated that the C isozymes did not disappear by the end of conjugation (see Figure 10: B × C, sample 6). In repeating this cross, it was carried out in the peptone medium, and the exconjugants were sampled as soon as possible after conjugation—or at about 25 fissions thereafter. Out of 16 pairs examined, 15 were B in phenotype, and one had all of the B and all of the C isozymes. The exconjugants from the same pair were similar in phenotype, including the exceptional pair (see Figure 15). This pair was subjected to an extensive analysis. A previous section documented the behavior of the two exconjugant clones during asexual reproduction. Now, what happens when these clones are bred?

In making crosses with these clones the crosses were carried out in the peptone medium, as described above. The exconjugants were not separated, but the entire exconjugant population was used in making the extracts. Later, from each pair, two randomly selected clones were carried to maturity in the bacterized medium and then retested after being put back on the peptone medium. In every case, each isolate from the same pair tested qualitatively the same as in the previous test of the pair culture. Hence, the kind of medium does not affect the results. Moreover, exconjugants from the same pair were probably alike. In crosses of "recovered" types (see below) the exconjugants were separated and were qualitatively similar in phenotype (with one exception) although quantitative variations were recorded. The results of the crosses made with the F_1 B+C clones are shown in Table 4.

In the first cross they were inbred (two caryonides of different mating type from the same pair were crossed) to produce an F_2, and in two separate crosses, the frequencies of exconjugant clones with the B, B+C and C groups of esterases were 5, 12, and 3, and 2, 11 and 5, respectively. The distribution of these numbers seemed to fit a good 1:2:1 ratio and suggested that a single locus might be involved in the control of the class I esterases. Under such an hypothesis each allele at this locus would determine a protein of different net charge—either B or C in type. Both proteins might be produced by a heterozygote. The isozymes, then, would arise by secondary modifications of these proteins as a result of nongenetic processes.

In the simplest form of this hypothesis, certain predictions were made for the backcross generations. These predictions were not met. Where a 1:1 ratio of B to B+C (or B only) was expected in the backcross to strain B, an 8:9:2 ratio of

TABLE 4

Distribution of class I esterases among the progeny of B+C clones

Cross	No. pairs tested	No. pairs with:		
		B group	C group	B+C groups
(B+C) × (B+C)	20	5	3	12
	18	2	5	11
	38	7	8	23
(B+C) × C	17	4	4	9
	17	6	5	6
	34	10	9	15
(B+C) × B	19	8	2	9

TABLE 5

Results of inbreeding F_2 progeny from a cross of (B+C) × (B+C)

Type of F_2 progeny:	No. pairs tested	B group	C group	B+C groups
B	8	8	0	0
C	8	0	8	0
B+C	12	5	2	5
B+C	8	1	4	3*

* Exconjugants were tested separately from each pair and in all pairs except one (here) the two exconjugants from the same pair were similar in phenotype. Here, the other exconjugant in one of the three pairs was "B" in type.

B to B+C to C was obtained. Similarly, the backcross to strain C yielded something that approached a 1:2:1 ratio of B to B+C to C where a 1:1 ratio of B+C to C was predicted. In both backcrosses the appearance of the nonparental phenotype was unexpected: C was not expected in the backcross to the B strain or B in the backcross to the C strain. Thus, all classes of offspring appeared in all of the crosses of the B+C hybrids.

A breeding analysis has been carried out on four of the F_2 progeny (from the inbreeding of F_1 B+C clones). Two of these were classified as B+C, one was classified as C and one was classified as B in phenotype. Table 5 shows that, when inbred, each of these classes bred "true". The two recovered "pure" types gave rise to only B or to C in their progeny. The two B+C clones gave rise to all three classes (B, B+C and C) in their progeny, repeating the behavior of their parents. In these crosses the exconjugants were separated, and, with one exception, those from the same pair were alike in phenotype. The exception occurred in one of the B+C crosses, and from one pair one exconjugant was B+C in type, the other being B. Although it is satisfying to be able to recover B's and C's which breed true, from a genetic viewpoint the critical crosses have not, as yet, been made. These are the crosses of clones with the nonparental phenotypes arising in the backcross generations. It is possible that these exceptions are actually B+C in genotype.

In summarizing the results of the breeding experiments it is difficult to put together all of the data into one harmonious scheme. The results of the original cross and the crosses of the "B" hybrids to strain C (Table 3) stand in contrast to the results of the crosses with the B+C clones (Tables 4 and 5). The first series of crosses gave rise to skewed ratios of B to B+C, and the C class was entirely missing from this pedigree. In the second series of crosses all three classes appeared—and in high frequencies.

Curiously enough, a similar pattern of behavior was manifested in these two series of crosses with regard to the presence or absence of mating type I (found in strains A, C and D, but not in strain B). At 30°C the frequency of mating type I in strain C is high (48 percent). At this temperature the frequency falls to about 20 percent in heterozygotes between *some* strain C clones and strain B; however, in heterozygotes between *other* strain C clones and strain B the frequency drops precipitously (zero to two percent). The strain C clone used as a parent in the present series of crosses yielded virtually no mating type I when crossed to the B strain, and, with two exceptions, mating type I was absent from the entire pedigree of the first series of crosses. The two exceptions appeared in subclones of two selfing B+C clones—in the F_1 and in the second backcross generation. In the second series of crosses, on the other hand, mating type I did appear. However, the distribution of mating type I was not random; it was confined to pairs which contained B+C or C class I esterases (Table 6). In no pair that was classed as B did mating type I appear. On the other hand, the mating types characteristic of strain B (IV, VII) were distributed among pairs with all three class I esterase phenotypes. These mating types also appear in high frequency in animals heterozygous for mating type alleles (mt^B/mt^C). It is possible that linkage between the mating type locus and a postulated esterase locus might explain this non random distribution of mating type I. A breeding analysis of the *capacity* of clones, differing in their esterases, to produce the various mating types will be necessary in order to test this possibility. An analysis of the four F_2 clones has been completed. The C clone and one of the two B+C clones gave rise to all mating types in their progeny and were thus heterozygous at the mating type locus (mt^B/mt^C). The B clone and the other B+C clone gave rise to no mating type I and may have been homozygous for the B mating type allele (mt^B/mt^B). It is clear, however, that mating type I and the B group of esterases are not

TABLE 6

Distribution of mating types I and IV (or VII) among pairs differing in class I esterases

Cross: \ Pairs:	B+C		C		B	
	I	IV	I	IV	I	IV
F_1 (B+C) × F_1 (B+C)	4/23*	12/23	2/8	3/8	0/7	5/7
F_1 (B+C) × C	3/15	6/15	4/9	3/9	0/10	5/10
F_1 (B+C) × B	1/9	4/9	2/2	0/2	0/8	6/8
F_2 (B+C) × F_2 (B+C)	1/5	2/5	1/2	0/2	0/5	4/5

* Of 23 pairs that were B+C, four showed mating type I, etc.

necessarily mutually exclusive since clones of strains A and D, which contain the B group, may manifest mating type I.

DISCUSSION

The application of histochemical techniques to proteins separated by starch gel electrophoresis has shown that variety 1 of *T. pyriformis* contains two classes of esterases: an eserine sensitive class (class I esterases) and an eserine insensitive class (class II esterases). Two groups of class I esterases are found, B and C, and each of these occurs in different inbred strains. Each group of esterases (B or C) contains four to five electrophoretically separated components (isozymes). The isozymes of each group show characteristic levels of activity which are elicited by specific growth conditions. An analysis of the inheritance of the *groups* of class I esterases during asexual and sexual reproduction has revealed the following:

(1) Each group of esterases (B or C) is maintained during asexual reproduction and after intrastrain crosses.

(2) When B is crossed with C, most of the progeny are B in phenotype, and none are C; however, a new class of clones appears in a very few of the hybrids—B+C.

(3) This distribution is repeated in successive backcrosses of "B" hybrids to C.

(4) No dissociation of B isozymes or of C isozymes is observed, although *all* the B and *all* the C isozymes may be produced by a single clone (B+C hybrids).

(5) Exconjugants from the same pair are alike in phenotype.

(6) Clones with both B and C present (B+C) show intraclonal variability, but the rate of change with fissions is low.

(7) B+C clones when inbred or backcrossed to the B or C strains give rise to all three classes (B, B+C and C) in their progeny.

(8) Further inbreeding of a few of the F_2 progeny from a cross of (B+C) × (B+C) has shown that the recovered "pure" clones (B and C) breed true. F_2 B+C clones give rise to all three classes (B, B+C and C) in their offspring.

(9) Mating type I, which is found in strain C but not in strain B, is absent from the B × C pedigree with the exception of two B+C clones. It is present in the offspring of B+C clones but only in progeny which are classed as C or B+C.

The breeding experiments show that the class I esterases are inherited, since the types put into a cross can be recovered in "pure" form in a later generation. Thus, clones differing in phenotype (B and C), when crossed, give rise to offspring which are usually B and occasionally B+C in type. The latter, when crossed, give rise, once more, to the B and C phenotypes, each of which breeds true. The details of these crosses, however, show inconsistencies with one another and rule out a simple genetic explanation.

Several hypotheses can be considered for the control of this system. None of these can be completely rejected at present, and certain aspects of each could be combined into a single hypothesis. The first to be considered is direct genic control of these esterases. The results of the breeding analysis preclude any

simple genetic explanation. However, paramutation (BRINK 1958) could be considered as operating in the crosses of "B" by C clones. Here, the C allele might be inactivated or transformed to B when combined with the B allele. The B+C phenotype might arise in clones in which paramutation had not taken place. The hypothesis runs into difficulty when trying to account for the persistence of effect in the backcross generations unless the ability to cause paramutation is also transmitted.

A second hypothesis which can be considered is particulate in nature. Two types of cytoplasmic particles could be postulated—*b* and *c*. The observation of intraclonal variability in clones containing both groups of esterases lends itself to such an interpretation. An hypothesis similar to that proposed for kappa in *Paramecium aurelia* (SONNEBORN 1959) might be envisioned, although here it might not be necessary to postulate genes in control of the particles. Some of the data support such an hypothesis. Difficulties are encountered when trying to account for the different results of crosses of clones with only *b* particles and of crosses of clones containing *b* and *c* particles.

A synthesis of the particulate hypothesis and a nuclear control system might be found in an hypothesis based upon some type of nucleo-cytoplasmic interaction analogous to that proposed for the mating type and serotype systems of variety 1 of *T. pyriformis* (ALLEN and NANNEY 1958; NANNEY 1959a; NANNEY and ALLEN 1959). In the case of the esterases, a single locus in control of the two groups would be postulated. Superimposed upon this control would be some form of epigenetic mechanism (NANNEY 1958), such as nuclear differentiation, which would be concerned with the expression of these enzymes. Unlike the mating type system in this variety, the exconjugants are alike in their esterase phenotype. Thus, nuclear differentiation must take place very early (ORIAS 1959) or under the influence of a cytoplasmic trigger in order to account for their conformity. If nuclear differentiation occurred in the presence of a cytoplasmic trigger and with cytoplasmic exchange, all the developing new macronuclei would receive the same stimulus, but heterogeneity could arise *within* the macronucleus, and the cytoplasmic trigger could affect the differentiation of the macronuclear subnuclei (ALLEN and NANNEY 1958; NANNEY 1959; NANNEY and ALLEN 1959). Usually the subnuclei would be triggered to differentiate in the B direction in a cross of B × C; but, if some of the subnuclei were differentiated to B and some to C, a heterogeneous macronucleus would result, and the clone would be B+C in phenotype. According to the ALLEN-NANNEY model, segregation of subclones phenotypically pure for B or pure for C should follow with subsequent fissions. A single subclone pure for C was observed. However, the rate of fixation was lower than that predicted on the basis of 45 subnuclei (SCHENSTED 1958).

The chief difficulty with this hypothesis, as with the particulate hypothesis, lies in explaining the difference in outcome of the cytoplasmic triggers in the crosses of "B" × C and in the B+C crosses. If paramutation occurred in the first series of crosses, it would mask the effect of nuclear differentiation, triggered by a cytoplasmic influence, which could operate in the second series of crosses. A combination of events might actually take place. It is hoped that future investi-

gation will reveal the mechanism or mechanisms controlling the inheritance of the esterases.

Whatever mechanism is involved in the control of the class I esterases, the significance of the isozymes should be mentioned. If, indeed, these esterases are under the control of a single locus, each allele must be responsible for several gene products. No dissociation of the isozymes from the group was observed although quantitative variations in their activity could be elicited by particular growth conditions. Whether the isozymes are interconvertible, as was the case with different polymers of bromocresol green (FRANGLEN and GOSSELIN 1958), is not known. If so, it might argue for one of the two possible structural arrangements discussed below.

Multiple gene products have been reported for the haptoglobins of man (SMITHIES and WALKER 1955) and the Beta-globulins of cattle (SMITHIES and HICKMAN 1958). Two interpretations of the observed multiplicity can be made with regard to protein structure (see ANFINSEN 1959, for a review of the 1 gene: 1 polypeptide hypothesis). The polypeptide produced by a gene could form a single protein, perhaps made up of other polypeptides produced by genes at other loci, and this single molecular species might be aggregated to form polymers of various size. Or, the polypepetide produced by a gene could be combined with several different polypeptides to form several different proteins. Recently, SMITHIES (1960) was able to resolve the protein portion of the human haptoglobins into a single molecular species for each allele by thioethanol and urea treatments, which would be expected to break SH-bonds. This result suggested that a single macromolecule is produced by each gene and that polymerization of these in various ways leads to the appearance of multiple gene products.

Similar treatments would be useful in deciding which type of arrangement occurs in Tetrahymena with regard to the esterases. In any event, the active site of the enzyme apparently is not affected by gene substitution, by polymerization, if a single macromolecule is involved, or by its combination with other gene products, if different macromolecules are involved. The ultimate biochemical basis of the class I esterases and the nature of their genetic and epigenetic control remains for the future to decide.

SUMMARY

The application of histochemical techniques to proteins separated by starch gel electrophoresis has shown that variety 1 of *Tetrahymena pyriformis* contains two classes of esterases: an eserine sensitive class (class I esterases) and an eserine insensitive class (class II esterases). Two groups of class I esterases (B and C) are found and each of these occurs in different inbred strains. Each group contains several electrophoretically distinct components or isozymes. The isozymes of each group show characteristic levels of activity which are elicited by specific growth conditions. Each group of class I esterases (B or C) is strictly inherited within a clone during vegetative reproduction and after conjugation within the strain. However, when clones with different groups of esterases are crossed, only the B phenotype is usually observed in the hybrid, although in about

one out of 25 of the hybrids both groups appear. Exconjugants from the same pair are alike in phenotype. This behavior is repeated in successive backcrosses of "B" hybrids to the C parent. In clones containing both groups of esterases (B+C) both groups are maintained with successive fissions, although intraclonal variation is observed in the level of activity of each group. When such clones are crossed, all three classes (B, B+C, and C) appear in the F_2 and in backcrosses to the two parents. The recovered "pure" clones in the F_2, when inbred, breed true, while the B+C F_2 clones, when inbred, give rise to all three classes of progeny. As a working hypothesis, it is suggested that a single locus may be involved in the control of the groups of class I esterases but that the expression of a particular group is influenced by paramutation and/or the interaction of a cytoplasmic trigger and nuclear differentiation.

ADDENDUM

Since submitting this manuscript, the apparent dilemma between the data of Table 3 and Tables 4 and 5 has been resolved. Two phenomena were confused. The C strain clone used as a parent in crosses (Tables 3 and 4) has been found to act as a leaky "genetic dud". Using normal members of the C strain, F_1 hybrids (B × C) are initially B+C in phenotype but with subsequent fissions "differentiate" to B or to C. No carry-over of this differentiation to subsequent generations occurs and good genetic ratios characteristic of a single locus are obtained.

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HETEROZYGOUS EFFECTS ON VIABILITY, FERTILITY, RATE OF DEVELOPMENT, AND LONGEVITY OF DROSOPHILA CHROMOSOMES THAT ARE LETHAL WHEN HOMOZYGOUS[1]

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THE purpose of this study was to determine whether "recessive" alleles that contribute to the concealed variability of natural populations of *Drosophila melanogaster* are completely recessive, or whether they have sufficient dominance that selection operates primarily on heterozygotes. The experiments consisted of extracting a series of recessive lethals and semilethals from a natural population and measuring their heterozygous effects on preadult mortality, rate of development, longevity, and fertility.

DOBZHANSKY and WRIGHT (1941) found that lethals were less frequent in natural populations of *Drosophila pseudoobscura* than would be expected from the mutation rate if the mutants were completely recessive and mating were at random in a large population. They concluded that this might be due to partial dominance or local inbreeding or both. Similar results were found by DUBININ (1946) in *D. melanogaster*.

STERN and NOVITSKI (1948) and STERN, CARSON, KINST, NOVITSKI and UPHOFF (1952) made a direct test by measuring viabilities of flies heterozygous for lethal-bearing X chromosomes. The lethals were partly spontaneous and partly X-ray induced. MULLER and CAMPBELL (unpublished, see MULLER 1950) did a similar study with second chromosome lethals induced by ultraviolet. The two studies agree in showing a 4–5 percent reduction in viability of the lethal-bearing heterozygotes during preadult stages.

These data are averages and do not rule out the possibility that there is a mixture of partially dominant, recessive, and even overdominant mutants. The latter two would accumulate to a far greater extent in a population than partial dominants. Hence, a study of heterozygous effects of lethals from a natural population would be especially sensitive in detecting whether most lethals are partially dominant. For such loci, the harmonic mean of the dominance in a group of new mutants should equal the arithmetic mean in an equilibrium population (MORTON, CROW, and MULLER 1956). The mean dominance at equilibrium, estimated in this way from the data of MULLER and CAMPBELL, is about 2–3 percent, but such an estimate is subject to a large error. However, the data to be reported here are in good agreement with this earlier crude estimate.

A similar study has been reported for *D. willistoni* (CORDEIRO 1952; PROUT

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1952). There is a clear indication of some deleterious effect of lethal heterozygotes from natural populations, but the number of chromosomes tested was too small and the internal consistency too low for any precise conclusion as to the amount of dominance. A preliminary report with similar conclusions has been published by DA CUNHA *et al.* (1958). See also GOLDSCHMIDT and FALK (1959).

Since preadult viability is only one component of total fitness, we have also investigated the developmental rate, longevity, and fertility of flies heterozygous for a lethal-bearing second chromosome.

A more extended report of some of these and other experiments is in preparation, to be presented by one of us (Y. HIRAIZUMI) as a doctoral thesis.

Isolation of lethal-bearing chromosomes

Drosophila melanogaster males, trapped from a wild population in Madison, Wisconsin, were crossed individually with a *Cy/Pm* female (*Cy* = Curly wing, with inversions in both arms of chromosome II; *Pm* = Plum eye color). A single male offspring of *Pm* phenotype was mated with a *Cy/Pm* female. From the progeny, a pair of *Cy/+* sibs were mated, and the F_3 progeny counted. If the chromosome being tested is normal, the expected proportion of non-Curly flies is one third since the homozygous Curly chromosome is lethal. Individual cultures were classified according to the percentage of wild type, and the results are summarized in Table 1.[2]

The combined frequency of lethals and semilethals (cultures with less than half the expected number of wild type flies) was 212/735, or about 29 percent. Comparable frequencies have been obtained by other workers.

Fifty of the lethal-carrying chromosomes were tested to see if they contained identical (or allelic) lethals, by making crosses between *Cy/+* flies carrying independent wild chromosomes. Of the possible 1200 tests, 1182 were completed, among which seven produced no wild type progeny. Thus, the probability of identity in two randomly chosen chromosomes in this population was about 0.6 percent. This is somewhat larger than that found by DUBININ (1946) in tests of flies from different areas, so there is probably some local inbreeding effect in our population.

Preadult viability

Fifty-three lethals, 64 semilethals, and 60 control strains were chosen at random and *Cy/+* males were mated with *cn/cn* females (*cn* = cinnabar eye). From the progeny, non-Curly males were selected and mated with *cn bw/cn bw* females (*bw* = brown eye; the double mutant *cn bw* has white eyes). For each chromosome tested, 20 replications were made in 25 × 95 mm shell vials, each containing two males and four females. Possible errors due to nonvirginity of the females could be recognized by the appearance of *cn bw* progeny. After 5–6 days

[2] Among the chromosomes examined, six (five lethal and one control) behaved in a strange way, giving 0.80–0.95 wild type flies. This turned out to depend on distorted segregation ratios in heterozygous males (SANDLER, HIRAIZUMI and SANDLER 1959). These chromosomes have been omitted from the data in this report.

TABLE 1

Frequencies of lethal (no wild type flies), semilethal (0.0–0.167 wild type), deleterious (0.167–0.250), normal (0.250–0.416) and super vital (more than 0.416) second chromosomes in a natural population of D. melanogaster

Date of collection	Percentage of wild type flies								Number of chromosomes tested
	0	0–8.3	8.3–16.7	16.7–25.0	25.0–33.3	33.3–41.6	41.6–50.0	50.0	
Sept. 13	4	7	4	9	28	8	2	0	62
Sept. 22	4	4	1	6	10	5	2	3	35
Oct. 2	3	15	4	12	35	17	8	8	102
Oct. 13–14	4	4	4	3	8	1	0	0	24
Oct. 17	15	16	9	30	54	15	0	0	139
Oct. 19	9	14	5	22	35	9	7	1	102
Oct. 20	12	34	8	28	67	27	7	2	185
Oct. 29	17	13	2	17	31	6	0	0	86
Total	68	107	37	127	268	88	26	14	735

Date	Percentage frequencies 0–16.7	16.7–25.0	25.0–41.6	41.6	Number
Sept. 13–22	24.7	15.5	52.6	7.2	97
Oct. 2–14	27.0	11.9	48.4	12.7	126
Oct. 17	28.8	21.6	49.6	0.0	139
Oct. 19	27.4	21.6	43.1	7.8	102
Oct. 20	29.2	15.1	50.8	4.9	185
Oct. 29	37.2	19.8	43.0	0.0	86
Total	28.8	17.3	48.4	5.4	735

in the first vial the parents were transferred to a second vial for an additional 5–6 days. The number of flies counted in each replication was from 70 to 150, averaging 115, so that about 2300 were counted for each tested chromosome.

The offspring of the cross, *cn bw/cn bw* female × *+/cn* male consist, theoretically, of half wild type and half cinnabar flies if the lethal-carrying chromosome has no effect in the heterozygote. Actually, the cinnabar flies are slightly less viable than their wild type sibs (SETO 1954); in this case the proportion of wild type flies in the control cultures is 0.5088. The average proportion wild type for each of the tested chromosomes is given in Table 2.

Since there were no values outside the range 0.4–0.6, the analysis of variance was performed on the original percentages without transformation. There was no significant difference between the lethal and semilethal classes ($F = 1.18$, $n_1 = 1$, $n_2 = 115$), so these are grouped in the subsequent analysis. The variance analysis in Table 2 shows a highly significant difference between the lethal + semilethal and the control groups.

The relative viability of the lethal + semilethal group was obtained by the formula $p'(1-p)/p(1-p')$, where p is the observed proportion of wild type flies in the control group and p' that in the lethal and semilethal group. The relative viability of the lethal and semilethal heterozygotes is 0.9693, or the selective disadvantage of these chromosomes relative to the normal is 0.0307.

TABLE 2

Proportion of wild type flies from the cross, cn bw/cn bw × cn/+, *where the + chromosome is lethal, semilethal, or normal when homozygous. Each value is the mean of 20 replications*

Lethals (53)				Semilethals (64)				Controls (60)			
0.467	.497	.510	.520	.439	.489	.500	.513	.472	.500	.510	.525
.467	.497	.511	.521	.452	.489	.501	.514	.475	.501	.511	.525
.475	.498	.511	.522	.472	.490	.503	.514	.478	.501	.512	.525
.476	.499	.511	.527	.475	.491	.505	.514	.478	.501	.514	.525
.476	.499	.512	.539	.476	.492	.505	.516	.484	.502	.515	.527
.478	.499	.512		.476	.493	.505	.516	.486	.502	.516	.528
.481	.499	.512		.479	.493	.505	.518	.486	.502	.516	.530
.483	.499	.514		.480	.494	.505	.518	.487	.503	.518	.530
.490	.499	.514		.482	.494	.506	.520	.494	.503	.518	.534
.493	.500	.514		.482	.494	.506	.523	.495	.503	.519	.534
.494	.502	.514		.484	.495	.506	.527	.496	.504	.520	.536
.494	.503	.516		.484	.496	.507	.527	.497	.506	.520	.545
.494	.503	.517		.485	.496	.508	.528	.498	.506	.520	
.496	.508	.519		.487	.498	.510	.528	.498	.506	.523	
.497	.510	.520		.488	.498	.511	.531	.498	.508	.524	
.497	.510	.520		.488	.498	.512	.549	.498	.508	.524	
Mean = .5026				Mean = .4997							
Mean = .5010								Mean = .5088			

Analysis of variance (lethals and semilethals grouped together)

	S.S.	D.F.	M.S.	F
Between lethal and control	.04799	1	.04799	8.38**
Between chromosomes within control	.31178	59	.00528	1.83**
Between chromosomes within lethals	.69041	116	.00595	2.25**
Between chromosomes	1.00219	175	.00573	
Within chromosomes (control)	3.29243	1140	.00289	
Within chromosomes (lethal)	5.88031	2223	.00265	

As stated earlier, the proportion of chromosomes containing lethals or semilethals in this population was 0.288. Assuming that these are distributed in Poisson fashion, the mean number of mutants among chromosomes with at least one is $m/(1-e^{-m})$, where e^{-m} is the number of normal chromosomes. The corrected selective disadvantage per lethal or semilethal locus is 0.0261. As stated earlier, this is in agreement with the value of 0.02–0.03 calculated indirectly from the data of MULLER and CAMPBELL (MORTON, CROW and MULLER 1956).

The variance among individual chromosomes is highly significant (Table 2), both in the control and in the lethal-semilethal classes. The variance is about 0.00030 in the lethal-semilethal and about 0.00026 in the control chromosomes, the former being slightly but not significantly larger. In the data of MULLER and CAMPBELL on mutants newly induced by ultraviolet, the variance was about 0.000083 in the control and 0.000133 in the lethal class. These differ significantly

from our results (in the control class, $F = 3.17$, $n_1 = 59$, $n_2 = 15$), ours being about three times as large. This is not surprising, for our chromosomes are a randomly chosen sample from a natural population whereas MULLER and CAMPBELL's material was from homogeneous laboratory strains. The difference between the lethal and the control group is 0.000050 in MULLER and CAMPBELL's data and 0.000034 in ours. This difference is not statistically significant, but if taken at face value it suggests that the natural population has lethal genes that are less diversified in their heterozygous effects. This is to be expected because of the more rapid elimination of those with the largest heterozygous effects.

Rate of development

In an organism where generations overlap, generation time is an important component of total fitness. Therefore, the developmental rate (period from egg laying till eclosion) was measured for a sample of the lethals, after a preliminary experiment suggested that the rate was slower than in the controls.

Twenty-eight lethal and 21 control chromosomes were chosen at random for testing. From each chosen line four replications of the cross 1 *cn*/+ male × 2 *cn*/*cn* females were made, and the females were allowed to oviposit for one day. Progeny counts were made at half-day intervals after the 9.5th day. Most of the cultures had 20–60 progeny, and those outside the range 20–80 were discarded to exclude any serious density-dependent effects.

The mean ratio of *cn*:+ for the lethals was 1.008 and for the controls, 1.020 (Table 3). The difference, though small, is highly significant ($F = 7.75$, $n_1 = 1$, $n_2 = 47$). (For the analysis of variance, the *cn*:+ ratio was computed for each culture and these were averaged for each chromosome. The apparent discrepancy in degree of freedom is caused by the pooling of tests on the same chromosome from different experiments.) Thus the lethals, on the average, have a develop-

TABLE 3

Rate of development in lethal heterozygotes and controls. In each case the measurements are made on the progeny of the cross, cn/cn × +/cn. *Experiments I, II, and III were done at different times*

	Number tested	Mean day of emergency +	Mean day of emergency *cn*	Ratio *cn*: +	Difference *cn* − +
Control					
Exp. I	14	10.99	11.20	1.019	0.21
Exp. II	16	10.30	10.54	1.023	0.24
Exp. III	6	11.02	11.15	1.012	0.13
Mean				1.020	0.21
Lethal heterozygotes					
Exp. I	12	10.99	11.06	1.006	0.07
Exp. II	16	10.40	10.55	1.014	0.15
Exp. III	14	11.09	11.14	1.004	0.05
Mean				1.008	0.09

mental rate about one percent slower than the controls. No tests were done on the semilethals.

Fertility

Measurement of female fertility is not difficult, for the number of fertile eggs laid at different ages can be readily measured. Male fertility is more of a problem, for a large difference in sperm production may not be significant from the standpoint of total progeny produced. The only measurement made in these studies was mating activity, which of course is only one component of total fertility.

Female fertility: Preliminary experiments suggested that larval crowding has a significant effect on adult fertility; so an attempt was made to grow the flies under as uniform conditions as feasible. Three or four parent females were allowed to oviposit for three days, then progeny were taken for test only if the total number was between 50 and 70. Two separate experiments were done.

In Experiment I, 24 control, 21 semilethal, and 21 lethal chromosomes were tested. One $+/cn$ female and three cn/cn males, all less than 24 hours old, were put in a vial. Transfers were made to fresh vials roughly every five or six days until egg laying stopped. New young males were added at approximately two-week intervals. Each chromosome was replicated ten times, except for a few cultures with only nine.

In Experiment II, 21 control, 21 semilethals, and 22 lethal chromosomes (not including any of those tested in Experiment I) were tested. The only difference in procedure was that transfers were made every four or five days.

The results are shown in Table 4. The two experiments differ significantly, presumably because of the different transfer interval and uncontrolled environmental factors. The amount of propionic acid used as a mold inhibitor was changed in the interval between the two experiments, and this may account for the large difference. However, there was not a significant interaction between experiment and class.

Although the results in Table 4 show a lower productivity of lethal and semilethal heterozygotes, the differences are not statistically significant, and the experiment is therefore inconclusive.

TABLE 4

Number of progeny flies per female parent. Each chromosome was replicated ten times

	Control	Semilethal	Lethal
Experiment I			
Mean number per female	248.7	237.3	217.7
Relative number	1.000	.954	.875
Experiment II			
Mean number per female	329.4	328.8	316.5
Relative number	1.000	.998	.961
Total relative number	1.000	.976	.918

Although there is not a significant difference in the total number of progeny produced, there is a highly significant difference in the age distribution of egg-laying activity (Table 5) (For the analysis of variance, ages of female were grouped into five, giving four degrees of freedom). The data are from Experiment II only, since precise age records were not kept in the first experiment. The control flies laid more eggs at an early age and fewer later than the lethal and semilethal heterozygotes. Thus, the fitness of the control females is significantly greater since earliness of reproduction is an important component of fitness.

Male fertility: Males from 21 control and 22 lethal strains were placed individually for 24 hours with three virgin females of age 1.5–2 days. In Experiment I the males were less than five hours old, in Experiment II they were 24 hours old, and in Experiment III they were three days old. In all other respects the experiments were alike, and the same strains were tested. The males were kept away from any females until the beginning of the test.

The results are shown in Table 6. In no case was the difference significant. Perhaps a more refined experiment with conditions adjusted to nearer 50 percent insemination or measuring competition between different males would have demonstrated a difference, but the present results are inconclusive.

Longevity

It is known that whether or not a female Drosophila is laying eggs has a definite effect on her longevity (BILEWICZ 1953; MAYNARD SMITH 1958), this being

TABLE 5

Mean number of progeny per female per day by age of the female (days after emergence). Data from Experiment II

Age of female	1–5	6–10	11–14	15–19	20–23	24–27	28–31	32–36	37–40
Control	9.75	10.93	12.49	9.74	9.26	7.28	4.37	3.78	2.11
Semilethal	8.49	8.36	10.77	7.96	7.89	7.84	6.44	6.14	5.45
Lethal	7.20	8.71	9.90	8.03	7.79	7.40	6.33	5.39	3.91

Analysis of variance

	S.S	D.F.	M.S.	F
Between class	549.96	2	275.0	<1
Between ages	186,047.2	4	46511.8	22.1**
Class–age interaction	16,818.7	8	2102.3	6.5**
Error		305	324.4	

TABLE 6

Mean proportion of females inseminated in three experiments

	Exp. I	Exp. II	Exp. III
Lethal	.758	.907	.960
Control	.757	.944	.958

inversely related to the amount of egg laying. Consequently, these experiments were done in two ways, with males and females together and with the sexes separated.

Experiment I, sexes separated: Twenty flies were collected within 24 hours of emergence from each of 39 control and 35 lethal lines. They were kept in groups of ten in a shell vial at 25°C. The flies were changed to fresh food every two days and counts were made every four days without etherization. A second experiment (I′) was identical except that only ten replications of each strain were made.

Experiment II, males and females together: For this experiment 24 control and 24 lethal chromosomes were tested. Ten flies from each strain were put with five *cn* flies of the opposite sex in a shell vial, and counts were made at eight-day intervals. Every 16 days young flies of the sex opposite to that being tested were added. The experiment was repeated once for females (II′).

The results are given in Table 7, and in Figures 1 and 2. There is a clear and statistically significant decrease in longevity in males heterozygous for a lethal chromosome in both experiments with sexes separate. On the other hand, when the males and females were together, the longevity was less for both lethal heterozygotes and controls but there was no significant difference between the two classes.

The results for female longevity show no significant difference between lethal heterozygotes and controls. This is true for both virgin and mated females, although the former live considerably longer than the latter in both lethal heterozygotes and controls. If we take the data of Table 4 at face value, despite the fact that the differences are not statistically demonstrable, and assume that lethal heterozygotes lay fewer eggs, we have a possible explanation of these results. Since nonlaying females live longer, it is possible that the greater potential longevity of the control flies is offset by the longevity-decreasing effect of greater and earlier egg production.

DISCUSSION

It is clear that lethal and semilethal alleles from a natural population have an appreciable deleterious effect in heterozygotes, an effect of sufficient magnitude

TABLE 7

Mean length of survival (from eclosion) in days for lethal heterozygotes and controls

	Males			Females			
	Sexes separate		Males with females	Sexes separate		Males with females	
	Exp. I	Exp. I′	Exp. II	Exp. I	Exp. I′	Exp. II	Exp. II′
Control	59.05	62.20	50.10	53.96	57.68	53.48	37.48
Lethal	55.66	59.52	51.00	54.46	58.49	51.85	40.43
Lethal/control	.943	.957		1.009	1.014	.970	1.079
	.950		1.018	1.012		1.024	

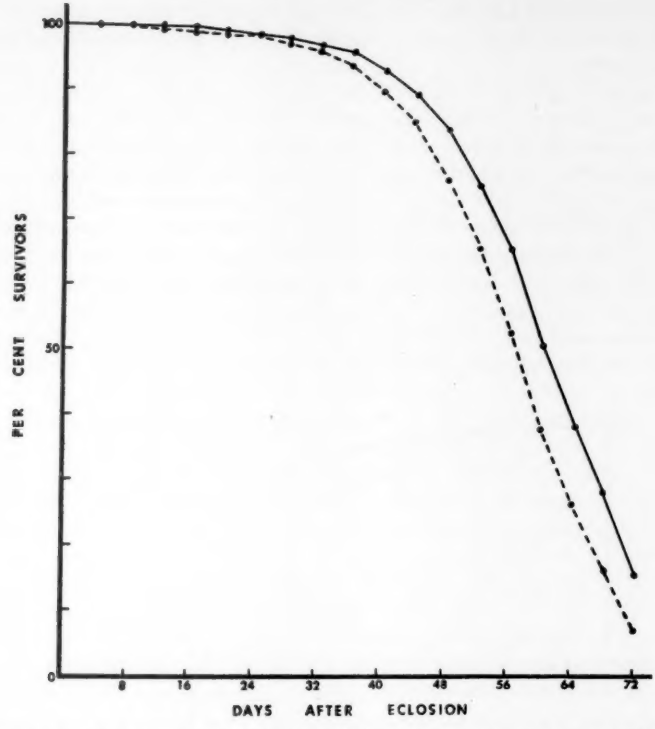

FIGURE 1.—Survival curve for males. The solid line is the control, and the broken line is the lethal heterozygotes.

that elimination of such alleles from the population must usually be in the heterozygous condition. The heterozygous effect on preadult viability is such as to decrease this by about 2.5 percent. The effect on rate of development, longevity of males, and age pattern of reproduction in females is also significant, but the data are not reproducible enough for a quantitative assessment. Probably other aspects of fitness, for which this study gave equivocal results, are also affected.

It is possible to make a crude estimate of the effect of all the measured factors on fitness. The delayed egg laying of lethal heterozygotes, as compared with the controls, is an important factor in fitness in an organism such as Drosophila with overlapping generations and rapid expansion during some parts of the year.

Because of different rates of maturation and age distribution of egg laying, fitness is best estimated with a continuous time parameter. FISHER's "Malthusian parameter," m, is defined by

$$\int_0^\infty l_x \, b_x \, e^{-mx} \, dx = 1$$

where l_x is the proportion of survivors at age x and b_x is the number of progeny per parent at age x. With biparental organisms b_x is half the number of progeny per pair of parents.

From the data of Tables 2, 3, and 5, the Malthusian parameter for lethal heterozygotes and control females can be computed roughly by numerical integration. Longevity makes little difference in total fitness, so is ignored. The Malthusian parameter as thus estimated is approximately 0.214 for lethal and semilethal heterozygotes and 0.224 for the controls, when time is measured in days.

Assuming a 14-day generation time, this corresponds to a selective difference, s, of about ten percent between the lethal heterozygotes and the controls. This has a large error, and is likely to be a gross overestimate for several reasons: (1) This is based on females and a corresponding difference in fertility of males has not been demonstrated. (2) There is no correction for multiple mutants in a single chromosome (except for the preadult viability component, which is de-

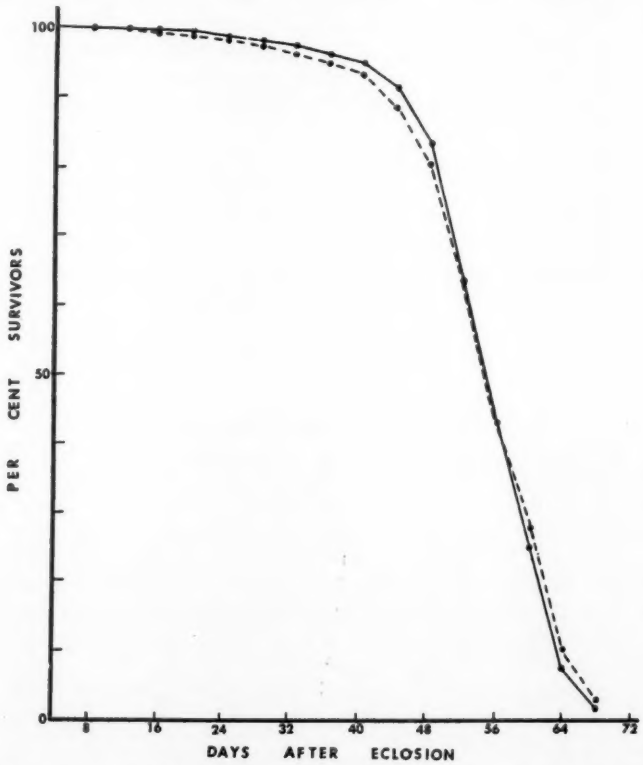

FIGURE 2.—Survival curve for females. The solid line is the control, and the broken line is the lethal heterozygotes.

termined with much more precision). (3) Probably of greater importance, the average number of progeny per pair of parents in nature when averaged over a long time cannot be greatly different from two, whereas we estimate m from a laboratory population where a single female produces several hundred progeny. The difference in total fitness of the two groups might have been much less, had these been measured under circumstances where the progeny number per parent was smaller. This would be especially true if intrabrood competition and density dependent factors were involved.

For these and other reasons, we cannot estimate with any precision the total effect of selection in heterozygotes from these data. However, it is clear that the lethal and semilethal heterozygotes are impaired in several adult fitness components, not just in preadult viability.

An independent estimate of s can be obtained from the frequency of lethal genes in the population and the known or assumed mutation rate, assuming random mating in a large population (DOBZHANSKY and WRIGHT 1941; PROUT 1952). By this procedure s is computed to be 0.029, in almost exact agreement with the value for the selective difference in preadult viability that we have obtained. With a Poisson correction, the value is 0.025. This kind of estimate depends on several assumptions (for example, the constancy of s among all lethals) which are not strictly correct, but it can serve as a rough confirmation.

In Table 1 the data show a progressive change in lethal and semilethal frequency with time. DUBININ (1946) has reported a similar change. The reason for this is not clear. It may be that the population is not at equilibrium between mutation and selection, or that there is migration from a neighboring population with a higher lethal frequency. Possibly the population becomes extremely small in the winter, during which time many lethals are eliminated as homozygotes, and the frequency rises steadily during the summer. However, our data are insufficient to test this notion.

A few instances of identity of lethals were discovered. These are the best candidates for the mildest deleterious effect or a beneficial effect in heterozygotes. However, none of these showed any tendency to have a higher preadult viability or total Malthusian parameter than the average of all the lethals. Hence there is no support for any appreciable number of heterotic lethals from our data.

Several investigators (STERN *et al.* 1952; MUKAI and BURDICK 1959) have discovered instances of lethals that almost certainly have a beneficial effect on fitness in heterozygotes, or simulate this through linked combinations. The fact that no clear evidence for heterotic lethals was found in our study does not, of course, demonstrate that they do not exist. A small minority may be present, and make a major contribution to the total fitness.

Consider a locus where one homozygote is lethal and the other has a disadvantage of one percent relative to the heterozygote.

Genotype	AA	Aa	aa
Frequency	p^2	$2pq$	q^2
Fitness	$1-s=0.99$	1	$1-t=0$

At equilibrium the frequency of the lethal allele will be $s/(s+t)$, or 0.01. A mutant of this frequency might well have been undetected in our experiments.

The effect of this locus is to cause a segregation load (CROW 1958) of 0.01 ($sp^2 + tq^2$); that is, to reduce the population fitness by about one percent in comparison to what it would be if all individuals were heterozygotes. On the other hand, 500 loci with partial dominance and an average mutation rate of 10^{-5} will cause the same load. This illustrates how a small minority of overdominant loci may still be a major factor in fitness of a randomly mating population.

With the above overdominant model, almost all the load is due to the mildly deleterious *AA* homozygotes and hardly any to the lethal *aa* class, for the *AA* load is sp^2 or about 0.01 while the *aa* load is tq^2 or 0.0001. Therefore, the lethal homozygotes are responsible for only one percent of the total random mating segregation load. This means that there will be a large selective pressure on modifiers that increase the viability of *AA* homozygotes and very little on those that modify the *aa* class. It would appear, then, that loci such as this would have a relatively short evolutionary history; they would be modified into "classical" loci, provided only that the requisite modifiers are available.

SUMMARY

Seven hundred thirty-five second chromosomes from a natural population were isolated and 212, or 29 percent, were lethal or semilethal when homozygous. There was no significant difference in the heterozygous effect of lethals and semilethals, but these collectively caused a reduction of 2.6 percent in preadult viability. The lethal heterozygotes also were slightly slower in developing. Females heterozygous for a lethal or semilethal produce fewer eggs at an earlier age than the controls although there was not a significant difference in the total lifetime production. The longevity of males heterozygous for lethals was slightly reduced, but there was no significant effect in females. In general, heterozygotes for lethals and semilethals from a natural population have an appreciable deleterious effect on preadult viability, and on components of adult fitness as well.

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CHROMOSOME MAPPING IN SACCHAROMYCES: CENTROMERE-LINKED GENES[1,2]

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AS organisms for genetic studies, yeast have certain advantages which include: a short generation time, several relatively stable states of ploidy, an ease of handling which permits the application of many of the techniques employed with bacteria, the possibility of a wide variety of biochemical studies, and the feasibility of tetrad analysis. To make use of these characteristics in certain types of experiments, chromosome maps are needed. This paper deals principally with one aspect of a wider program of chromosome mapping of Saccharomyces, the use of centromere-linked genes to estimate the chromosome number and provide a coherent framework for future studies of linkage.

MATERIALS AND METHODS

Of the 140 genes now identified in our yeast stocks, we have restricted ourselves to those loci which show centromere linkage and to a few others which are common to several breeding stocks and are of possible interest to other workers in the field of yeast genetics. They are listed in Table 1, together with their source, when appropriate, and with references. More complete characterizations of the phenotypes for the genes controlling the fermentation of the sugars are given by WINGE and ROBERTS (1948); LINDEGREN (1949); DOUGLAS and CONDIE (1954); and HAWTHORNE (1955a). CHEN, EPHRUSSI and HOTTINGUER (1950) described the original genic petite strain. A phenotype similar to film formation was described by MAGNI (1949). The isolation of the nutritional mutants is reported by EPHRUSSI, HOTTINGUER and TAVLITSKI (1949); POMPER and BURKHOLDER (1949); REAUME and TATUM (1949); and MORTIMER, LERNER and BARR (1957).

The strains used were heterothallic. Diploid hybrid clones were obtained by pairing haploid cells (CHEN 1950), by isolating single zygotes from a mating mixture, or by the prototroph-selection technique (POMPER and BURKHOLDER 1949). Originally, the gypsum slant technique described by LINDEGREN and LINDEGREN (1944) was used to promote spore formation, and only linear four-

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TABLE 1

The sources of the genes included in this report

Gene symbol	Phenotype	Strain	Received from	Reference
a	Mating type	93–3P	C. C. LINDEGREN	LINDEGREN 1949
α	Mating type	93–1C	C. C. LINDEGREN	LINDEGREN 1949
ad_1	Adenine requiring (pink)	99R	E. L. TATUM	REAUME and TATUM 1949; LINDEGREN 1949; ROMAN 1956
ad_2	Adenine requiring (red)	276/3br	B. EPHRUSSI	EPHRUSSI *et al.* 1949
ad_6	Adenine requiring (white)	1742A	H. ROMAN	ROMAN 1956
ad_8	Adenine requiring (white)	A15–55	H. ROMAN	
ar_4	Arginine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
CU_1	Copper resistance		original isolate (DCH)	HAWTHORNE 1955a; BRENNES-POMALES *et al.* 1955; LINDEGREN *et al.* 1959
Fl	Film formation		original isolate (DCH)	HAWTHORNE 1955a; MAGNI 1949
g_1	Galactose nonfermenter	1426A	C. C. LINDEGREN	LINDEGREN 1949; DOUGLAS and CONDIE 1954; HAWTHORNE 1956
g_2	Galactose nonfermenter	93–3P	C. C. LINDEGREN	DOUGLAS and CONDIE 1954; HAWTHORNE 1956
g_3 (g_8)	Galactose nonfermenter	186–2	O. WINGE	WINGE and ROBERTS 1948; HAWTHORNE 1956
g_4	Galactose nonfermenter	3352	A. P. JAMES	
hi_1	Histidine requiring	1914	C. RAUT	LINDEGREN and LINDEGREN 1951; LINDEGREN *et al.* 1959
hi_2	Histidine requiring		original isolate (DCH)	HAWTHORNE 1955a
hi_4	Histidine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
hi_6	Histidine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
is_1	Isoleucine requiring		original isolate (RKM)	MORTIMER *et al.* 1957; ROMAN and JACOB 1958; LINDEGREN *et al.* 1959

TABLE 1—(Continued)

The sources of the genes included in this report

Gene symbol	Phenotype	Strain	Received from	Reference
le$_1$	Leucine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
ly$_1$	Lysine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
ly$_2$	Lysine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
MA$_3$	Maltose fermenter		original isolate (DCH)	HAWTHORNE 1955a
MEL	Melibiose fermenter	CXL–10	C. C. LINDEGREN	LINDEGREN 1949; HAWTHORNE 1955a
me$_1$	Methionine requiring	67	S. POMPER	POMPER and BURKHOLDER 1949
me$_2$	Methionine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
p$_1$	Cytochrome deficient	B–26	B. EPHRUSSI	CHEN *et al.* 1950
p$_2$	Cytochrome deficient		original isolate (RKM)	MORTIMER *et al.* 1957
s-me$_1$	Suppresses *me*$_1$		original isolate (DCH)	
SU$_1$	Sucrose fermenter	93–3P	C. C. LINDEGREN	LINDEGREN 1949; HAWTHORNE 1955a
thr$_1$	Threonine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
thr$_2$	Threonine plus methionine or homoserine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
thr$_3$	Threonine plus methionine or homoserine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
thr$_4$	Threonine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
tr$_1$	Tryptophane requiring	62–20–194	S. POMPER	POMPER and BURKHOLDER; HAWTHORNE 1955b
tr$_2$ *(an)*	Tryptophane requiring	1914	C. RAUT	LINDEGREN and LINDEGREN 1951; LINDEGREN *et al.* 1959
tr$_5$	Tryptophane requiring		original isolate (RKM)	MORTIMER *et al.* 1957
ur$_1$	Uracil requiring	62–20–194	S. POMPER	POMPER and BURKHOLDER 1949
ur$_3$	Uracil requiring		original isolate (RKM)	MORTIMER *et al.* 1957; LINDEGREN and LINDEGREN 1951; LINDEGREN *et al.* 1959

spored asci were dissected (HAWTHORNE 1955b). After the discovery of several centromere markers which simplified the analysis of unordered tetrads, it was more expeditious to utilize one of the various sodium acetate sporulation media (STANTIAL 1935; ADAMS 1949; FOWELL 1952), which yield principally oval (unordered) asci. A recent improvement in dissection technique has resulted from the use of snail digestive juices to digest the ascus wall (JOHNSTON and MORTIMER 1959). Only the data from complete tetrads are reported in this paper.

Nutritional requirements were assayed on synthetic media based on the one proposed by WICKERHAM (1946). Copper resistance was scored on the synthetic medium containing 1 mM/l. of copper sulfate (BRENNES-POMALES, LINDEGREN and LINDEGREN 1955). Fermentation tests were conducted in Durham tubes, in Kahn tubes, with brom-cresol-purple as a *p*H indicator, or on agar (two percent) with brom-thymol-blue as the indicator. The medium in each case was one percent peptone, one percent yeast extract, and one percent of the diagnostic sugar. The mating type of auxotrophic segregants was determined by the production of diploid prototrophs in crosses to haploids of known mating type and with complementary nutritional requirements. Otherwise, the determinations were based on the direct observation of zygotic figures. It was possible to score two or more complementary genes controlling either the fermentation of a sugar or a nutritional requirement using techniques described previously (HAWTHORNE 1956; ROMAN 1956).

Centromere linkage: Tetrad analysis of four-spored asci of Saccharomyces hybrids makes possible the location of a gene with respect to the centromere of its chromosome. Since centromeres of homologous chromosomes segregate at the first division of meiosis, a gene closely linked to the centromere will be expected to show a high frequency of first division segregation. The frequency of second division segregation is, therefore, a measure of the recombination frequency between the gene and the centromere. For genes closely linked to the centromere, the recombination frequency is one half the frequency of second division segregation. For genes with a greater interval, the recombination frequency must be calculated from equations which deal with the effects of multiple crossovers (BARRATT, NEWMEYER, PERKINS and GARNJOBST 1954).

When the relationship of the spores in the ascus is known, gene-centromere linkage can be determined directly. If the relationship of the spores cannot be ascertained from their position in the ascus and if known centromere markers are absent, it is necessary to use an indirect method for the estimation of the second division segregation frequency (LINDEGREN 1949; PERKINS 1949; and WHITEHOUSE 1950).

The asci from Saccharomyces hybrids are of two morphological types: (1) oval asci in which spore relationship is undeterminable, and (2) elongated asci in which the spores are arranged in a linear series. An investigation of the four-spored linear asci obtained from a diploid hybrid revealed that spores with non-sister nuclei alternate in the elongated ascus (HAWTHORNE 1955b). This conclusion was based primarily on data concerning the segregation of a single gene, tr_1, controlling the requirement for tryptophane. The spore arrays for trypto-

phane independence in 74 asci were as follows: 1(++−−), 1(+−−+), 2(−++−), and 70(+−+−). The relegation of 70 out of 74 asci to a single spore array was interpreted as evidence that the fourth array, (+−+−), is the noncrossover class and that the gene tr_1 is closely linked to the centromere. On this basis, the gene tr_1 was mapped at 2.5 units from the centromere since about five percent of the asci were in the second division segregation classes: (++−−), (+−−+), and (−++−).

Further dissections of linear asci provided evidence for nuclear slippage in some of the asci. The compilation of the spore array data for tr_1 from 515 asci is as follows: 9(++−−), 39(+−−+), 28(−++−), and 439(+−+−). If the noncrossover class is strictly confined to the fourth array, the number of asci with the first array (++−− or −−++) should be equal to the total number of asci with the second and third arrays. The unexpectedly high proportion of asci with the second and third arrays suggests that in five to ten percent of the asci, slippage has occurred and the sister nuclei are located together in the center and at the ends of the elongated ascus; some of the second and third arrays should therefore be included in the noncrossover class.

The error in the scoring of the asci for second division segregation, introduced by the occasional slippage of the nuclei may be contended with in two ways: (1) The frequency of asci with the first array, (++−−), may be used to obtain an estimate of second division segregation, since this array would be obtained in one half the cases of second division segregation whether slippage occurs or not. (2) A correction of the scoring of individual asci with evidence of nuclear slippage is also possible when a decision can be based on several independently segregating genes with close centromere linkage (HOWE 1956). For example, if in a given ascus each of the genes tr_1, ad_1, and g_1 (1, 5, and 8 units from their respective centromeres) segregated as either (+−−+) or (−++−), it would be safe to assume that these arrays represent the noncrossover class for this ascus since the probability of simultaneous second division segregation followed by a central-terminal assortment of these three loci is 0.00004.

The second procedure was the one generally followed for the determination of second division segregation. The data on the distribution of the spore arrays in linear asci from crosses in which it was possible to correct for the slippage of the nuclei are given in Table 2. Since this method of determining the first division segregation pattern in individual asci can also be applied to the unordered tetrads of oval asci, it was no longer necessary to dissect linear asci when the crosses involved several centromere markers. The data from oval asci on the segregation of the centromere linked genes are given in Table 3.

The genes listed in Tables 2 and 3 are divided in three groups on the basis of their second division segregation (SDS) frequencies: (1) SDS frequencies significantly less than 66.7 percent, (2) SDS frequencies about 66.7 percent, (3) SDS frequencies significantly higher than 66.7 percent. Centromere linkage is indicated by an SDS frequency that is significantly less than 66.7 percent. An SDS frequency of 66.7 percent is the upper limit predicted with no interference and unlimited crossing over between the locus and the centromere (MATHER

TABLE 2

Centromere linkage data from linear asci, corrected spore arrays

Locus	++−−	+−−+	−++−	+−+−	Total	Percent second div. segregation
g_3	1	1	0	83	85	2.4
tr_1	6	4	3	412	425	3.1
ad_1	13	7	10	230	260	11.5
g_1	27	14	11	256	308	16.9
FI	45	21	16	329	411	19.9
α	70	48	35	365	518	29.5
hi_2	39	21	28	147	235	37.5
MA_3	101	50	50	134	335	60.0
hi_1	74	46	44	91	255	64.4
MEL	149	60	74	126	409	69.2
ur_1	120	69	51	107	347	69.3
tr_2	121	59	39	86	305	71.8
me_1	151	68	69	103	391	73.7
g_2	57	50	31	45	183	75.4
ad_2	105	46	31	55	237	76.9

1935). Therefore, the SDS frequencies significantly higher than 66.7 percent are interpreted as evidence for chiasma interference and, moreover, indicate that the genes in question are close enough to the centromere for the effects of interference to be manifested. An SDS frequency in the neighborhood of 66.7 percent may mean either that the gene is located so far from the centromere that interference does not prevent the occurrence of multiple crossovers or that the gene is linked to the centromere by an interval of about 36 to 41 units (see Figure 3).

An examination of Tables 2 and 3 shows that there are 19 genes close enough to the centromere to be of value in this study; the degree of linkage ranges from one unit (tr_1, g_3) to 33 units (me_2). The centromere-linked genes were included in crosses in various combinations to determine the number of independent linkage groups and, thus, the number of chromosomes.

Linkage relationships of the centromere-linked genes: For the determination of a minimal chromosome number on the basis of the number of centromere markers, methods must be adopted for establishing nonlinkage as well as for detecting linkage. By establishing nonlinkage we mean a demonstration that two genes are not linked to the same centromere. With tetrad data obtained from a diploid of composition *A B/a b*, the statistics to be employed are the frequencies of parental ditype (PD) asci (*AB AB ab ab*), nonparental ditype (NPD) asci (*Ab Ab aB aB*), and tetratype (T) asci (*AB Ab aB ab*). The following criteria were adopted: (1) Linkage is indicated when the PD:NPD ratio is significantly in excess of 1:1, provided the prospective map distance between two centromere-linked genes is equal to either the sum or the difference of the gene-to-centromere distances. (2) Nonlinkage is indicated when the NPD:T ratio exceeds 1:4 (PERKINS 1953) or

if the recombination value for the genes under consideration is greater than the sum of the gene-to-centromere distances.

Consistent map distances are essential for the demonstration of linkage since occasionally tetrad ratios indicative of both linkage and nonlinkage are encountered. There are two phenomena which may give rise to this situation: (1) A preferential segregation of non-homologous centromeres or affinity (MICHIE and WALLACE 1953) can give a false indication of linkage. (2) Chromatid interference across the centromere resulting in an excess of four-strand double crossovers can give a false indication of nonlinkage.

When dealing with genes showing only slight centromere linkage, one may obtain tetrad ratios which are not indicative of either linkage or nonlinkage. However, some information can still be gained under these circumstances if, on the basis of consistent map distances, the possibility of locating the genes on the same chromosome arm can be eliminated. Examples illustrating these conditions and the resolution of the problems will be given below.

TABLE 3

Centromere linkage data from oval asci

Locus	First-division segregation	Second-division segregation	Percent second-division segregation
tr_1	895	4	0.45
le_1	850	39	3.9
ur_3	153	16	9.5
ad_1	629	70	10.0
p_1	293	34	10.4
g_1	454	82	15.3
ar_4	449	85	15.9
hi_2	332	151	31.3
tr_5	132	61	31.9
hi_6	207	100	32.5
α	650	432	39.9
hi_4	152	110	42.0
thr_1	189	144	42.3
ly_1	71	85	54.5
ad_6	175	234	57.3
g_4	68	92	57.5
me_2	110	163	59.7
ly_2	82	134	62.0
SU_1	103	169	62.2
ad_8	92	166	64.5
thr_3	76	143	65.4
MA_3	166	337	67.0
me_1	94	209	69.0
g_2	85	237	73.5
MEL	149	415	73.5
CU_1	71	224	76.0

In Table 4, there is presented a summary of all tetrad analyses which are indicative of linkages involving centromere-linked genes. Of the 19 centromere-linked genes (Tables 2 and 3), 15 show linkage either with one another or with genes not directly established as centromere-linked. There are nine genes in the latter category with SDS frequencies near 66.7 percent and higher. For the genes not listed in Tables 2 or 3, the SDS frequencies that were observed in samples of about 50 asci are as follows: thr_4 58 percent, $s\text{-}me_1$ 67 percent, is_1 69 percent, and p_2 74 percent. The determination of gene sequence and map distances will be deferred until later in this paper.

Throughout this study, crosses were made specifically to gather evidence for nonlinkage for those centromere markers which appeared to be segregating independently. The first cross in this series gave diploids heterozygous for the centromere markers tr_1, g_1, *FI*, hi_2, and α, as well as MA_3, *MEL*, ur_1, me_1, and ad_2. Table 5 gives the data from 75 tetrads which had 2:2 segregations for all five of the centromere-linked genes. (Tetrads with irregular segregations, i.e., segregations deviating from the expected 2:2 ratio, were observed for most of the loci with a frequency on the order of one to two percent. To maintain a homogeneous sample, these asci are not included in the presentation of the segregation data for individual hybrids, Tables 5 through 9. However, data from these asci on the loci with 2:2 segregations were utilized in the determination of centromere linkage and the analysis of multiple crossovers, Tables 2, 3, 12, and 13.) Nonlinkage for each of the centromere-linked genes, tr_1, g_1, *FI*, α, and hi_2, was established with

TABLE 4

Linkage data for the centromere-linked genes

Gene pair	PD	NPD	T
g_1–ly_2	43	6	95
hi_6–ly_1	75	8	130
me_2–p_2	50	0	12
α–hi_4	97	7	174
α–thr_4	25	0	12
tr_1–g_2	70	0	0
tr_1–$s\text{-}me_1$	17	1	32
le_1–tr_5	226	0	61
le_1–ad_6	125	6	175
tr_5–ad_6	32	12	62
p_1–ar_4	163	0	18
ar_4–thr_1	213	0	62
p_1–thr_1	119	0	52
thr_1–CU_1	106	0	111
p_1–CU_1	60	8	164
ur_1–thr_3	64	1	105
thr_3–hi_1	64	0	1
hi–is_1	49	0	20
is_1–tr_2	56	0	13
hi_1–tr_2	118	2	95

TABLE 5

*The analysis of hybrids No. 1111 and No. 1113: data from 75 tetrads with 2:2 segregations for all the genes in question**

	tr_1	g_1	FI	α	hi_2	MA_3
tr_1		36:26 13	34:34 17	32:28 15	35:23 27	17:12 46
g_1	8.9 43.3		29:21 25	27:28 20	24:22 29	12:16 47
FI	11.8 43.3	17.9 44.7		27:29 19	21:20 34	12:12 51
α	10.3 47.3	16.4 50.7	19.3 51.3		21:18 36	11:15 49
hi_2	18.9 48.7	25.0 48.7	27.9 49.3	26.4 48.0		9:19 47
MA_3	34.0 46.7	40.1 52.7	43.0 50.0	41.5 52.7	50.1 56.7	
Number second div. seg.	2	11	15	13	25	46
Percent second div. seg.	2.7	14.7	20.0	17.4	33.3	61.2
Centromere distance $k = 0.1$	1.4	7.5	10.4	8.9	17.5	32.6

* In the upper portion of the grid, the numbers of parental (PD) and nonparental (NPD) ditypes and the tetratypes (T) are given as $\frac{\text{PD:NPD}}{\text{T}}$ for the various combinations. In the lower portion of the grid, the summed centromere distances of the two genes being compared are given over the observed recombination values.

the observation that the NPD:T ratios for each pair was significantly greater than 1:4. The comparison of the summed centromere distances with the recombination values supported this conclusion. In every case, the recombination value (a minimal representation of the distance between the loci in the case of linkage) greatly exceeded the sum of the centromere distances.

From the second cross, a hybrid was obtained which was heterozygous for the following genes: tr_1, ad_1, g_1, *FI*, α, hi_1, MA_3, SU_5, MG_2 (alpha-methyl glucoside fermentation), *s-me*$_1$, ur_1, *MEL*, ad_2, and *can* (canavanine resistance). An analysis of the data from 50 tetrads, Table 6, supports the independent segregation of the centromere markers, tr_1, ad_1, g_1, *FI*, and α. Linkage between tr_1, and *s-me*$_1$ was indicated by a $\frac{\text{PD:NPD}}{\text{T}}$ ratio of $\frac{17:1}{32}$. Since there was no crossing over between tr_1 and the centromere in these tetrads, the one NPD ascus indicates a single four-strand double exchange occurred in the interval between *s-me*$_1$ and the centromere. This result gave promise that linkage between a gene with a similar SDS frequency (64 percent) and any of the markers showing strong centro-

TABLE 6

*The analysis of hybrid No. 1249: data from 50 tetrads with 2:2 segregations for all the genes in question**

	tr_1	ad_1	g_1	FI	α	hi_1	MA_3
tr_1		24:17 / 9	21:20 / 9	20:23 / 7	13:19 / 18	10:11 / 29	11:7 / 32
ad_1	9.3 / 43.0		23:11 / 16	13:22 / 15	9:15 / 26	13:5 / 32	8:6 / 36
g_1	9.3 / 49.0	18.6 / 38.0		13:14 / 23	14:17 / 19	10:10 / 30	9:8 / 33
FI	7.2 / 53.0	16.5 / 59.0	16.5 / 51.0		13:15 / 22	13:10 / 27	7:11 / 32
α	19.0 / 56.0	28.3 / 56.0	28.3 / 53.0	26.2 / 52.0		10:10 / 30	9:14 / 27
hi_1	31.0 / 51.0	40.3 / 42.0	40.3 / 50.0	38.2 / 47.0	50.0 / 50.0		14:9 / 27
MA_3	34.8 / 46.0	44.1 / 48.0	44.1 / 49.0	42.0 / 54.0	53.8 / 55.0	65.8 / 45.0	
Number second div. seg.	0	9	9	7	18	29	32
Percent second div. seg.	0	18	18	14	36	58	64
Centromere distance $k = 0.1$	0	9.3	9.3	7.2	19.0	31.0	34.8

* See footnote, Table 5.

mere linkage, tr_1, le_1, etc., through ar_4, would not go undetected. Therefore, the genes hi_1 and MA_3, considered to be loosely linked to the centromere, are included in Table 6. The PD:NPD ratio of 13:5 for hi_1-ad_1 is suggestive of linkage; however, subsequent investigations have shown that hi_1 and ad_1 are not linked and that hi_1 is located on the chromosome marked by ur_3 (later in this paper). Of the remaining genes, MA_3, SU_5, and MG_2 are closely linked (no crossovers in this sample of 50 asci) while the others segregate independently from each other and the centromere markers.

Two strains differing in the centromere-linked genes tr_1, le_1, ad_1, p_1, g_1, ar_4, hi_2, tr_5, α, hi_4, thr_1, and ad_6 were crossed to give hybrid X714, Table 7. Irregular segregations for the ad_1 locus, with the tetrads distributed as 57 (4:0), 9 (3:1), and 31 (2:2), suggested that the hybrid was trisomic for the chromosome carrying this gene (therefore, AD_1 AD_1/ad_1). None of the remaining centromere markers or the other genes present, MA_3, ad_8, g_2, CU_1, and MEL, gave irregular

ratios indicative of location on this chromosome. The assortment of the centromere markers tr_1, le_1, p_1, g_1, hi_2, and either α or hi_4 met the criteria for nonlinkage. The $\frac{23:5}{55}$ ratio for α and hi_4 is a significant demonstration of linkage. From a comparison of the recombination value (38) with the centromere distances for α (20 units) and hi_4 (24 units), the genes were located on the opposite sides of the centromere.

Of the remaining centromere markers, tr_5 and ad_6 are linked to le_1; and ar_4, thr_1, together with CU_1, are linked to p_1 (Table 4). Following an examination of the crossover patterns in individual asci, the sequence tr_5, le_1, centromere, and ad_6 was adopted (later in this paper) while the genes p_1, ar_4, thr_1, and CU_1 were placed in that order on the same chromosome arm. Although the distal gene of the latter group, CU_1 showed 70 percent SDS in this cross, only a single four-strand

TABLE 7

*The analysis of hybrid X714: data from 81 tetrads with 2:2 segregations for all the genes in question**

	g_1	α	hi_4	tr_1	hi_2	le_1	p_1
g_1		23:22 36	20:23 38	28:40 13	18:33 30	36:31 14	40:27 14
α	29.7 49.3		23:3 55	24:23 34	16:28 37	23:25 33	19:25 37
hi_4	33.0 51.8	46.3 37.8		22:20 39	14:18 49	17:27 37	17:23 41
tr_1	8.8 57.4	22.1 49.3	25.4 48.8		33:27 21	39:37 5	41:35 5
hi_2	21.1 59.2	34.4 57.4	37.7 52.5	13.5 46.3		35:27 19	22:35 24
le_1	10.7 47.0	24.0 51.2	27.3 56.1	3.1 48.8	15.4 45.0		35:38 8
p_1	10.7 42.0	24.0 53.7	27.3 53.7	3.1 46.3	15.4 58.0	5.0 51.8	
Number second div. seg.	13	33	38	1	20	4	4
Percent second div. seg.	16.0	40.7	46.9	1.2	24.7	4.9	4.9
Centromere distance $k = 0.1$	8.2	21.5	24.8	0.6	12.9	2.5	2.5

* See footnote, Table 5.

TABLE 8

*The analysis of hybrid No. 1295: data from 50 tetrads with 2:2 segregations for all the genes in question**

	tr_1	le_1	p_1	ad_1	g_1	α	hi_2
tr_1		22:25 / 3	40:8 / 2	26:18 / 6	21:23 / 6	18:11 / 21	15:21 / 14
le_1	3.0 / 53.0		20:26 / 4	27:16 / 7	14:27 / 9	13:13 / 24	17:18 / 15
p_1	2.0 / 18.0	5.0 / 56.0		27:16 / 7	20:22 / 8	17:10 / 23	15:19 / 16
ad_1	6.1 / 42.0	9.1 / 39.0	8.1 / 39.0		20:18 / 12	9:14 / 27	15:17 / 18
g_1	6.1 / 52.0	9.1 / 63.0	8.1 / 52.0	12.2 / 48.0		15:13 / 22	17:15 / 18
α	22.2 / 43.0	25.2 / 50.0	24.2 / 43.0	28.3 / 55.0	28.3 / 48.0		17:9 / 24
hi_2	14.6 / 56.0	17.6 / 51.0	16.6 / 54.0	20.7 / 52.0	20.7 / 48.0	36.8 / 42.0	
Number second div. seg.	0	3	2	6	6	21	14
Percent second div. seg.	0	6	4	12	12	42	28
Centromere distance $k = 0.1$	0	3	2	6.1	6.1	22.2	14.6

* See footnote, Table 5.

double crossover and four other multiple exchanges were observed in the centromere-CU_1 interval. In contrast, a $\frac{28:12}{53}$ ratio for tr_5-ad_6 reflects a surprisingly high frequency of four-strand double exchanges occurring in this region spanning the centromere; four-strand exchanges account for more than half of the double crossovers observed (Table 12).

The observation of a barely significant PD:NPD ratio, 28:12, for tr_5-ad_6 gave warning that with the customary analysis one might miss detecting linkage between two genes similarly situated but a little more loosely linked to the centromere. A more sensitive analysis to detect trans-centromere linkage utilizes only the tetrads which have first division segregations for both of the genes under consideration (CATCHESIDE 1951). Under the hypothesis that the genes in question are on the opposing arms of the same chromosome: (1) all noncrossover tetrads must be of the first division segregation class, and (2) all NPD tetrads showing first division segregation must have resulted from a four-strand double crossover within one of the gene-centromere intervals. For example, if, with the above data,

only the ditype asci showing first division segregation are examined, the new PD:NPD ratio for tr_5-ad_6, 21:0, is highly significant evidence for linkage.

With the fourth cross in this series, there was obtained a hybrid, No. 1295, yielding asci with 2:2 segregations for the following centromere markers for the seven chromosomes predicted with the previous cross: tr_1, p_1, le_1, ad_1, g_1, hi_2, and α. The data from 50 tetrads presented in Table 8 supports the interpretation of nonlinkage for these genes. Regular segregations were observed for these additional markers: ar_4, thr_1, me_2, SU_1, ad_8, ur_2, ur_1, ad_2, MEL, MA_3, and CU_1. Again as shown in the previous cross, the genes p_1, ar_4, thr_1, and CU_1 proved to be linked. The rest of the genes segregated independently from each other and the centromere markers.

TABLE 9

*The analysis of hybrid X709: data from 65 tetrads with 2:2 segregations for all the genes in question**

	ad_1	g_1	α	tr_1	hi_2	le_1	hi_6	ar_4	ur_3
ad_1		27:24 14	15:18 32	26:30 9	19:21 32	33:20 12	14:22 29	21:28 16	30:27 8
g_1	11.0 47.6		11:21 27	26:32 7	22:19 24	30:24 11	20:20 25	21:29 15	33:22 10
α	25.8 52.3	24.2 53.1		12:29 24	15:16 34	23:14 28	11:16 38	14:20 31	17:20 28
tr_1	7.0 53.1	5.4 54.6	20.2 63.0		21:19 25	26:33 6	17:26 22	29:25 11	31:29 3
hi_2	25.8 49.4	24.2 47.6	39.0 50.8	20.2 48.4		21:14 30	15:19 31	16:19 30	20:17 28
le_1	10.2 40.0	8.6 45.3	23.4 43.1	4.6 55.4	23.4 44.6		20:21 24	19:31 15	25:34 6
hi_6	23.2 56.1	21.6 50.0	36.4 53.8	17.6 57.0	36.4 53.1	20.8 50.8		17:19 29	19:23 23
ar_4	14.2 55.4	12.6 56.2	27.4 54.5	8.6 46.9	27.4 52.3	11.8 59.2	24.8 51.5		23:28 14
ur_3	9.4 47.6	7.8 41.5	22.6 52.3	3.8 48.4	22.6 47.7	7.0 56.9	20.0 53.1	11.0 53.8	
Number second div. seg.	8	6	24	1	24	5	21	10	4
Percent second div. seg.	12.3	9.2	37.0	1.5	37.0	7.7	32.3	15.4	6.2
Centromere distance $k = 0.1$	6.3	4.7	19.5	0.7	19.5	3.9	16.9	7.9	3.1

* See footnote, Table 5.

The tetrad ratio observed for p_1-tr_1 $\frac{(40:8)}{2}$ is an anomaly incompatible with either independent segregation or linkage. Since nonlinkage between tr_1 and p_1 was clearly indicated by the analysis of hybrid X714, this result was interpreted as evidence for the preferential segregation of non-homologous centromeres. One other PD:NPD ratio, 14:27 for le_1-g_1, differed significantly, at the five percent level, from 1:1; however, from the 21 combinations of the seven centromere markers listed in Table 8, one chance deviation from 1:1 at this level of significance is not surprising.

The last cross in this series provided evidence for at least nine different chromosomes in Saccharomyces. The analysis of 65 tetrads from hybrid X709, Table 9, shows that two new centromere markers ur_3 and hi_6 segregated independently from the following genes marking the seven centromeres definitely established with the previous cross: tr_1, le_1, g_1, ad_1, ar_4, hi_2 and α.

Four more genes hi_4, thr_3, g_2, and *MEL* were segregating in the asci of hybrid X709. Further evidence for linkage between hi_4 and α was obtained with a $\frac{24:2}{39}$ ratio for this gene pair. Linkage between thr_3 and ur_3 was indicated by a $\frac{28:0}{37}$ ratio. There was no suggestion of linkage between g_2 and *MEL* and any of the other markers.

There was no evidence of preferential segregation of non-homologous centromeres in this cross. In contrast to the results obtained in the preceding cross, the centromeres marked by tr_1 and p_1 (in this case ar_4) segregated as 1:1 for parental and nonparental combinations. In Table 9, there is one PD:NPD ratio that does deviate significantly from 1:1 at a one percent level, the ratio of 12:29 for tr_1–α. However, the PD:NPD ratio of 15:21 for tr_1 and hi_4, located across the centromere from α, is not significant. By utilizing the results with both hi_4 and α to determine the segregation of this centromere relative to the one marked by tr_1, a PD:NPD ratio of 25:37 is obtained which again is not significant evidence for the preferential segregation of these centromeres.

Two groups of genes remain to be considered: (1) those genes closely linked to a gene already established as centromere linked will be dealt with in the next section; (2) those genes showing so little centromere linkage as to have been of little use in the early phases of this problem will be considered below. The genes in the latter category have SDS frequencies ranging from 54.5 percent for ly_1 to 64.5 percent for ad_8 (see Table 3). In some cases, the numbers of tetrads analyzed are insufficient to demonstrate a significant deviation from the 66.7 percent SDS expected with no centromere linkage. Even a significant deviation from 66.7 percent SDS might not necessarily be conclusive evidence for centromere linkage since it is conceivable that a SDS frequency in this range might be the consequence of a pronounced chromatid interference favoring two-strand or four-strand double crossovers.

TABLE 10

Tetrad analysis of the data from miscellaneous crosses involving the centromere markers and the genes showing slight centromere linkage

	ad_1	g_1	a	tr_1	ur_3	hi_2	le_1	p_1	hi_6
ly_1	19:26 / 53	13:18 / 37	48:86 / 187	10:16 / 39		4:4 / 10	22:17 / 43	10:13* / 22	75:8 / 130
ly_2	6:12 / 33	43:6 / 95	31:33 / 115	20:22 / 70	5:4 / 21	11:12 / 65	42:23 / 115	37:17 / 81	
g_4	10:15 / 37	11:8 / 29	16:13 / 87	41:25 / 90	10:11 / 33	14:15 / 50	19:20 / 78	14:10 / 25	7:11 / 36
me_2	21:24 / 90	27:23 / 83	42:42 / 153	37:47 / 128	11:16 / 41	23:20 / 63	19:19 / 82	24:30 / 92	9:13 / 42
SU_1	26:24 / 81	25:23 / 82	37:43 / 167	33:29 / 92	13:7 / 28	15:14 / 63	32:34 / 117	39:24 / 104	17:17 / 63
MA_3	49:45 / 171	45:48 / 230	68:57 / 261	38:34 / 172	10:7 / 37	35:32 / 125	48:42 / 203	15:21 / 79	34:25 / 129
ad_8	13:26 / 74	26:31 / 110	43:49 / 161	42:42 / 150	15:8 / 30	34:30 / 110	28:25 / 111	42:33 / 133	3:3 / 12

* The gene ar_4 marked the centromere of chromosome VIII in the analysis with ly_1.

† The numbers of parental (PD) and nonparental (NPD) ditypes and the tetratypes are given as $\frac{\text{PD:NPD}}{\text{T}}$.

Table 10 gives the data concerning the segregation of these genes of questionable centromere linkage, ly_1, g_4, me_2, ly_2, SU_1, MA_3, and ad_8 with respect to the centromere markers of the nine chromosomes established with hybrid X709. Two cases of linkage are evident: ly_1–hi_6 $\frac{75:8}{130}$ and ly_2–g_1 $\frac{43:6}{95}$. A $\frac{41:25}{90}$ ratio for g_4–tr_1 differs significantly from 1:1. However linkage between g_4 and tr_1 is unlikely, provided g_4 is actually centromere linked, since this ratio would require four-strand double crossovers greatly in excess of the numbers expected on the basis of the frequency observed for what would be a comparable region, tr_1–*s*-me_1 (see the discussion of hybrid 1249). The tetrad ratios show that me_2 is segregating independently from the nine centromere markers. Further evidence supporting centromere linkage for me_2 and thus indicating nonlinkage with these nine centromere markers will be given later. The three loci with a little more tenuous indication of centromere linkage, SU_1, MA_3, and ad_8, are also segregating independently from these nine centromere markers.

In a further attempt to locate these new markers, an analysis was made of their segregations with respect to each other and the following genes which show a similar order of centromere linkage: ly_2 chromosome II, hi_1 chromosome V, ad_6 chromosome VII, CU_1 chromosome VIII, and ly_1 chromosome IX. If one accepts these genes as being centromere linked, it is readily apparent from the data in

Table 11 that they all must be located on different chromosome arms. The one $\frac{\text{PD:NPD}}{\text{T}}$ ratio indicative of linkage, $\frac{23:11}{72}$ for g_4–CU_1 is attributed to the statistical fluctuation expected in a table of these dimensions since g_4 showed no link-

TABLE 11

Tetrad analysis of the data from miscellaneous crosses involving the genes showing slight centromere linkage

	g_4	SU_1	ad_8	MA_3	ly_2	hi_1	ad_6	CU_1	ly_1
me_2	30:21 / 81	15:20 / 91	17:21 / 54	38:37 / 136	18:22 / 55	8:7 / 29	11:9 / 39	23:33 / 107	3:3 / 28
g_4		10:7 / 46	19:12 / 50	9:13 / 54	7:4 / 35	5:8 / 14	15:17 / 56	23:11 / 72	8:4 / 12
SU_1			17:9 / 61	19:28 / 77	6:7 / 26	15:8 / 44	7:11 / 41	14:21 / 75	2:9 / 33
ad_8				25:28 / 108	1:5 / 14	1:2 / 15	25:23 / 100	30:28 / 114	
MA_3					11:9 / 58	28:25 / 67	10:11 / 44	20:14 / 87	24:18 / 50

[a] The numbers of parental (PD) and nonparental (NPD) ditypes and the tetratypes are given as $\frac{\text{PD:NPD}}{\text{T}}$.

TABLE 12

Multiple crossovers in regions spanning the centromere

Linkage group	Distribution of tetrads		Distribution of double crossovers: 2:3:4-strand double exchanges	Expected double crossovers k = 1.0	coincidence
III	no c.o.	85			
	c.o. I	75			
I II	c.o. II	75			
hi_5–c–α	c.o. I and II	43	12:24:7	50	0.86
		278			
VII					
I II III	no c.o.	21			
tr_5–leu_1–c–ad_6	c.o. I	6			
	c.o. II	2			
	c.o. III	41			
	c.o. I and II	0			
	c.o. I and III	20	6:4:10	21.3	1.08
	c.o. II and III	3	1:0:2		
		93			
IX	no c.o.	67			
	c.o. I	36			
I II	c.o. II	83			
hi_6–c–ly_1	c.o. I and II	27	8:11:8	32.5	0.83
		213			

TABLE 13

Crossovers in adjacent regions of linkage group VIII
I II III IV
c–p_1–ar_4–thr_1–CU_1

Distribution of tetrads*		Distribution of double crossovers:
no c.o.	33	2:3:4-strand double exchanges
c.o. I	5	4:3:2 + 1 (2 or 4 strands)
c.o. II	13	
c.o. III	21	
c.o. IV	63	
c.o. I and III	1	
c.o. I and IV	1	
c.o. III and IV	6	
c.o. II, III and IV	1	
	144	

Region	Crossovers	Map length	Locus	Centromere distance	Second div. segregation Number	Second div. segregation Percent
I	7	2.4	p_1	2.4	7	4.8
II	14	4.9	ar_4	7.3	21	14.6
III	29	10.0	thr_1	17.3	47	32.6
IV	71	24.7	CU_1	42.0	105	72.9

* Data from hybrids 1295 and X714.

age to the centromere marker of chromosome VIII, p_1 (see Table 10). The PD:NPD ratios for me_2–g_4, g_4–ad_8, and ad_8–SU_1 deviated enough from 1:1 to warrant an analysis of the first division segregation tetrads to check for trans-centromere linkage. The restricted PD:NPD ratios for me_2–g_4 (13:11), g_4–ad_8 (11:6), and ad_8–SU_1 (5:4) did not support this possibility of linkage. Thus, the establishment of nonlinkage for me_2, g_4, SU_1, MA_3, ad_8 and the centromere markers of the first nine chromosomes depends only upon the verification of centromere linkage for these markers. To date the evidence for centromere linkage justifies only the inclusion of me_2 on the chromosome maps as the centromere marker of chromosome X.

Chromosome maps: The results of the individual crosses presented in the preceding section can now be summarized by the construction of chromosome maps, Figure 1. Ten chromosomes are included in these maps; their centromeres are marked by the genes ad_1, g_1, α, tr_1, ur_3, hi_2, le_1, p_1, hi_6, and me_2. All genes established as being linked to the above centromere markers are represented on the maps. Distances were computed using the mapping functions of BARRATT *et al.* (1954), Figure 3. Except for the loci on chromosome VIII, the calculations are based on the data presented in Tables 2, 3, and 4. For the mapping of the loci on chromosome VIII, a more homogeneous sample, the data obtained from hybrids 1295 and X714 (Table 13) is used. The procedure followed to determine gene-centromere sequence will be illustrated below in the placement of the centromere in the linkage group tr_3–le_1–ad_6.

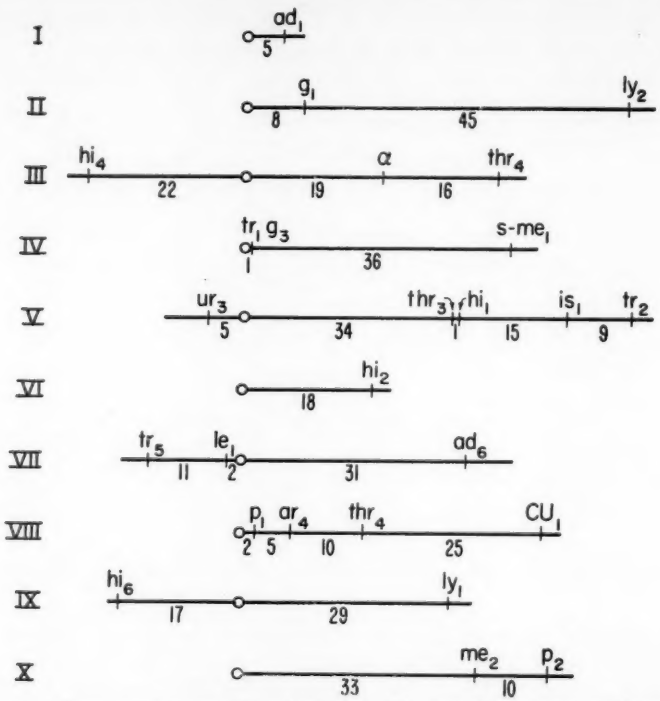

FIGURE 1.—Chromosome maps of Saccharomyces. The map distances in centi-morgans are derived from the second division segregation frequencies in Tables 2 and 3 and the tetrad ratios in Table 4.

The three genes, tr_5, le_1, and ad_6 gave 2:2 segregation in 93 of the tetrads analyzed in the third cross. Among these tetrads, there were five with second division segregations for le_1. The centromere segregation in these five tetrads was determined by the segregation of the genes tr_1, p_1, and g_1 so there was no doubt as to the occurrence of a crossover between le_1 and the centromere. Figure 2 gives the segregation patterns for the three loci and the crossover configurations that are needed under the hypotheses that the centromere is located (1) between le_1 and ad_6 and (2) between tr_5 and le_1. Only three segregation patterns were observed in these asci. Two asci are represented by the array in the first row. To obtain this segregation of the three loci, two crossovers would be required under the first hypothesis compared to four under the second. There were two asci with the second array, and again the first hypothesis is favored; only one crossover needs to be postulated if the centromere is between le_1 and ad_6, while three are required if it is between tr_5 and le_1. In the ascus with the last array, two crossovers are required under either hypothesis. On the basis of these results, the sequence tr_5–le_1–c–ad_6 was adopted (c indicates centromere).

The sequences c–g_1–ly_2 chromosome II, ur_3–c–thr_3 chromosome V, c–p_1–ar_4 chromosome VIII, and hi_6–c–ly_1 chromosome IX were determined from similar analyses. A comparison of the SDS frequencies and recombination values was sufficient to order the loci hi_4–c–α–thr_4 on chromosome III and c–me_2–p_2 on chromosome X. However, support for the latter order and the centromere linkage of me_2 came from an examination of the asci that were tetratype for me_2–p_2 which showed that for the most part me_2 had segregated in the first division as would be expected with the expression of chiasma interference as demonstrated in chromosome VIII (see later in this paper). The arrangement of the genes on chromosome IV, c–tr_1–g_3–s-me_1, is provisional since no asci with a crossover between g_3 and tr_1 have been recovered and only first division segregations have been observed for tr_1 in the crosses involving g_3 and s-me_1 (see Table 4).

An analysis of the crossover pattern in individual tetrads was also needed to establish the sequence of the closely linked genes on chromosome V. The order of thr_3 and hi_1 with respect to the centromere or the other markers was not determined until the analysis of data on 40 asci from a hybrid heterozygous for ur_3, thr_3, hi, and tr_2, made available through the courtesy of Dr. F. Sherman, gave us a tetratype tetrad for thr_3 and hi_1. In this ascus parental ditype segregations were observed for ur_3–thr_3 and hi_1–tr_2 thus clearly favoring the order ur_3–thr_3–hi_1–tr_2. The summary of the tetrad ratios for is_1 with hi_1 and tr_2 (Table 4) includes data from the work of Roman and Jacob (1958). A crossover analysis as well as a comparison of the recombination values places is_1 between hi_1 and tr_2.

Interference and the distribution of multiple crossovers: Preliminary to the selection of a mapping function, information was sought on the occurrence of chiasma or chromatid interference for both regions spanning the centromere and those restricted to one arm. Data on the crossovers in three regions in the first

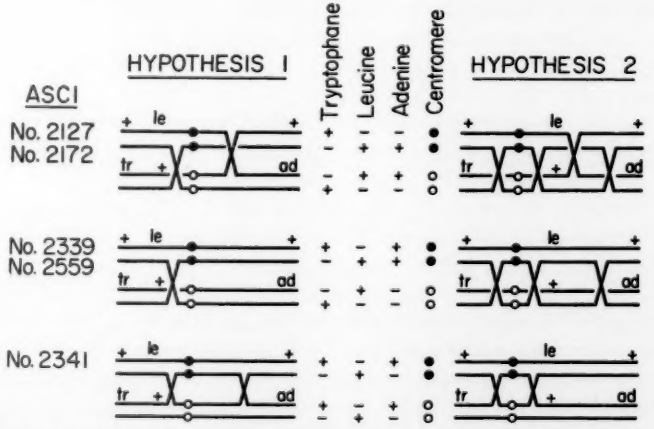

Figure 2.—Examples of the segregation patterns for tr_5, le_1, ad_6, and the centromere, showing the consequences of the alternative positions of le_1 with respect to the centromere.

category, chromosome III α–c–hi_4, chromosome VII tr_5–le_1–c–ad_6, and chromosome IX hi_6–c–ly_1, are presented in Table 12. None of the three regions gave convincing evidence of chiasma interference for crossovers in opposing arms. Ratios of observed to expected double crossovers of 43/50, 23/21.3 and 27/32.5 were obtained for the gene–centromere–gene intervals of chromosomes III, VII, and IX, respectively. All double crossovers were classified as to two-strand, three-strand or 4-strand exchanges to detect any indication of chromatid interference, *i.e.*, any deviation from the 1:2:1 ratio of two-, three-, and four-strand doubles expected on the classical model of crossing over. For chromosome III, the double crossovers are distributed as 12 two-strand, 24 3-strand, and 7 four-strand, in agreement with the 1:2:1 ratio. For chromosome VII, the 7:4:12 distribution is a significant departure from the 1:2:1 ratio expected. The 8:11:8 distribution for chromosome IX is again compatible with the 1:2:1 ratio. The summed frequencies for the three chromosomes, 27 2-strand, 39 3-strand, and 27 four-strand, fit satisfactorily the frequencies expected with the 1:2:1 distribution predicted with no chromatid interference.

Among the linkage groups on one arm, the loci c–p_1–arg_4–thr_1–CU_1 embraced the only region that was long enough to give information on the occurrence of double crossovers, yet contained markers spaced so that the chance of a double exchange occurring within an interval was negligible. Data from the analyses of hybrids 1295 and X714 on the crossovers which occurred within this region are presented in Table 13. There were 144 tetrads available where all the genes in question gave 2:2 segregations. The numbers of crossovers observed in the four intervals progressing from the centromere were 7, 14, 29, and 71. If, with the locus thr_1, we divide this region into two, the effects of chiasma interference are readily apparent. The ratio of observed to expected number of asci having crossovers in both 'halves' was 8/24. A more precise evaluation of the degree of chiasma interference was obtained through the following analysis of the data. The asci were classified according to tetrad rank or the number of crossovers for the entire region (WEINSTEIN 1936). In the 144 asci, there were 33 tetrads of rank 0 (no exchange), 102 of rank 1 (a single exchange), eight of rank 2, and one of rank 3. For no chiasma interference, the relative frequency of tetrads of different rank can be predicted from the Poisson distribution (BARRATT *et al.* 1954). Corresponding to the observed frequency of the tetrads of rank 0 (33 per 144 asci), one would expect 49 tetrads of rank 1, 35 of rank 2, 18 of rank 3, and nine of ranks higher than 3. The lack of agreement between the observed distribution and the numbers expected, if the crossovers occurred independently, indicates the influence of a strong positive chiasma interference. To obtain a useful expression of the degree of chiasma interference, use was made of the relation derived by BARRATT *et al.* 1954, (equation 7) to estimate tetrad rank frequency for different degrees of chiasma interference. In this relationship, the degree of interference is specified by the value of a coefficient 'k'. For no interference, k=1. For complete interference, k=0. Since a high degree of chiasma interference was indicated, tetrad rank distributions were calculated for k=0.2 and k=0.1. Corre-

sponding to the 33 rank 0 tetrads observed and with k=0.2, one would expect 94 tetrads of rank 1, 14 of rank 2, two of rank 3 and one of ranks higher than 3; while for k=0.1, the expected distribution is 103 tetrads of rank 1, 7.5 of rank 2, and 0.5 of rank 3. It can be seen that the values predicted with k=0.1 fit very closely the observed frequencies in all the ranks.

Evidence for some variation in the degree of interference for the above region in chromosome VIII came from the analysis of 56 asci from miscellaneous crosses which in some cases were lacking the markers p_1 or ar_4. The summed data, with a distribution of ten tetrads of rank 0, 39 of rank 1, and seven of rank 2, were indicative of a lesser degree of interference than that observed with hybrids 1295 and X714.

The choice or deriviation of a mapping function requires a knowledge regarding the strand relationships in multiple exchanges, i.e., the occurrence of chromatid interference, as well as the extent of chiasma interference. The data concerning multiple exchanges in one arm are again confined to the c–p_1–ar_4–thr_1–CU_1 region. In the above analyses, the multiple exchanges were distributed as six two-strand, five three-strand, and three two- or four-strand. Although this distribution conforms more closely to a 1:1:1 ratio, it is not a significant deviation from the 1:2:1 ratio expected with no chromatid interference. This distinction poses no problems when mapping centromere linked genes. However, for mapping loci with SDS frequencies significantly higher than 67 percent, a distinction between the two distributions becomes of importance due to the accumulation of multiple exchanges. Curves of the SDS frequencies based on both the 1:1:1 and 1:2:1 ratios of two-strand, three-strand, and four-strand exchanges for k=0.1 and k=0.2 are given in Figure 3. The frequency of NPD asci are also plotted for the four cases. By selecting the interference level and strand distribution which best fits the observed $\frac{\text{PD:NPD}}{\text{T}}$ ratio, one can obtain an estimate of the map distance between loosely linked genes.

DISCUSSION

Of the 140 mutant loci now in our breeding stocks about half have been investigated for centromere linkage. Twenty-seven genes had SDS frequencies of less than 67 percent (Tables 2 and 3). Twenty-two of these genes can be placed in ten different linkage groups (Figure 1). Only two of the remaining genes, *FI* and g_4, gave SDS frequencies that deviated significantly from 67 percent. Satisfactory scoring for the character film formation was obtained only in the highly inbred stocks used in the earlier experiments; for this reason the gene *FI* has not been included in the final maps. Although it is likely that g_4 marks the centromere of another chromosome, it is not presently included in the chromosome maps since the criteria for nonlinkage has not been satisfied for all the genes marking the centromeres of the first ten chromosomes, Table 10. With the accumulation of more tetrads showing the segregation of SU_1, ad_8, and MA_3, it is expected that

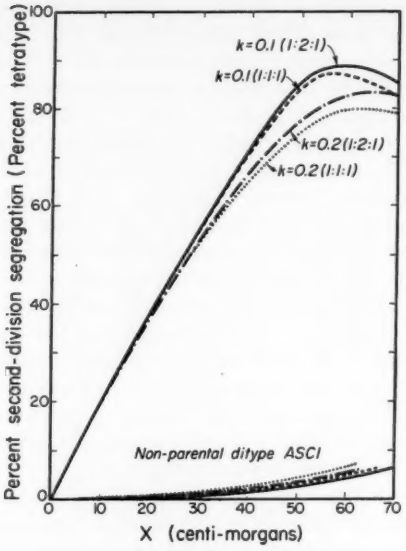

FIGURE 3.—Tetrad mapping functions (based on the derivation of BARRATT, NEWMEYER, PERKINS, and GARNJOBST 1954). Percent tetratype and nonparental ditype asci as a function of map distance for different degrees of chiasma and chromatid interference. The degree of chiasma interference is indicated by the value of k ($k = 1.0$, no interference, $k = 0$, complete interference). Chromatid interference is represented by the distribution of two-strand, three-strand, and four-strand multiple exchanges (in parentheses).

some of these genes will prove to mark the centromeres of additional chromosomes.

In the designation of the chromosomes, an attempt was made to follow the numbering of the chromosomes listed by LINDEGREN and LINDEGREN (1951). They present maps of five chromosomes with the following centromere markers: (I) *ad-1*, (II) *g*, (III) *α*, (IV) *pb* (paraminobenzoic acid), and (V) *ur*. However, the use of the gene *pb* to mark the centromere of chromosome IV is not justified from the analysis of the published data (Table 24–3, LINDEGREN 1949), since, as PERKINS (1953) has pointed out, the $\frac{\text{PD:NPD}}{\text{T}}$ ratio of $\frac{17:5}{45}$ for *pb–α* is actually indicative of linkage at a 99 percent level of significance. The four remaining chromosomes can be recognized. The genes marking the centromeres of chromosomes I, II, and III are common to our stocks: ad_1 was received from DR. E. L. TATUM and *g* (*g-1*) and *α* were received from DR. C. C. LINDEGREN. The uracil gene marking chromosome V was presumed to be ur_3 because of similar linkage relationships with hi_1 and the centromere (LINDEGREN *et al.* 1959). For chromosome IV, we have chosen the linkage group with the centromere markers tr_1 and

g_3. The remaining centromere markers designate chromosomes in the order in which independent assortment was established.

In a recent summary of linkage studies by LINDEGREN, LINDEGREN, SHULT, and DESBOROUGH (1959), there are listed seven and possibly nine linkage groups associated with centromeres. No data were furnished concerning the centromere linkage of the markers identifying these linkage groups: *CH* (choline), *UR*, *TH* (thiamine), *CU*, *GA*, α, *AD*, *PR* (proline), and *DX* (dextrin fermentation). However, four of the chromosomes correspond to linkage groups I, II, III and V in this study and in the earlier maps presented by LINDEGREN and LINDEGREN (1951) although their designation has been revised. We have decided to adhere to the original designations for reasons of priority and because of the rather speculative nature of their latest maps. For example, 'chromosome I' marked by *CH*, *UR*, and *TH* is presented as either a chromosome with three branches or a complex of chromosomes with absolute affinity of parental centromere combinations.

The centromere distances indicated in the earlier reports of LINDEGREN and LINDEGREN (1949, 1951) for the markers ad_1 11 units, g_1 six units, α 24 units, and *ur* five units agree well with the averages of our own determinations: ad_1 five units, g_1 eight units, α 19 units, and ur_3 five units. No attempt has been made to present confidence limits for the gene centromere intervals since for some genes, there appears to be considerable variation from cross to cross. The most exaggerated variation was observed for the mating type locus which showed only slight centromere linkage in the earlier crosses—58 percent SDS for the 74 tetrads of hybrids 1002, 1003, and 1004 (HAWTHORNE 1955b)—but was more closely linked in the hybrids of this study 17.4 percent SDS hybrids 1111 and 1113, 36 percent SDS hybrid 1249, 42 percent SDS hybrid 1295, 40 percent SDS hybrid X714, and 37 percent SDS hybrid X709. Two genes that have been established as being more loosely linked to their centromeres, hi_1 and CU_1, have also shown a significant variation in SDS frequency. With these loci the SDS frequencies have varied from 55 percent to 75 percent.

The above variation in SDS frequencies may be a reflection of changes in the interference constant as well as the map distance. An indication of this for the c–thr_1–CU_1 region of chromosome VIII came from the analysis of multiple exchanges in asci from miscellaneous crosses where the interference was less pronounced than that observed for hybrids X714 and 1295. Moreover the very strong interference demonstrated for chromosome VIII in hybrids X714 and 1295 is not necessarily applicable to the other chromosomes. For instance, with chromosome II, the $\frac{43:6}{95}$ ratio for g_1–ly_2, 62 percent SDS for ly_2, and the order c–g_1–ly_2 cannot be reconciled with a strong chiasma interference and a 1:2:1 distribution of two-strand, three-strand, and four-strand double exchanges. Since changing the postulated sequence of the loci to g_1–c–ly_2 requires nearly a doubling of the number of multiple exchanges between g_1 and ly_2, it seems more probable that it is the interference constant which is at variance with that observed for chromosome VIII.

SUMMARY

Extensive linkage studies in Saccharomyces were undertaken with more than 70 genes controlling nutritional requirements, fermentation of sugars, resistance to metals, and colonial characters. The emphasis was on the comparison of centromere linked markers, detected through tetrad analysis, to obtain an estimation of the minimal haploid chromosome number. Seventeen genes that showed a significant degree of centromere linkage were compared and found to fall into ten linkage groups which segregated independently. Another nine genes showing significant linkage to the centromere markers were placed on the maps of these chromosomes. There were five more genes with at least a suggestion of centromere linkage (a second division segregation frequency less than 67 percent) which remain unmapped.

An analysis of chiasma and chromatid interference was made for four of the linkage groups. Chiasma intereference was not observed in three groups comprised of regions spanning the centromere, while a coincidence value of 0.34 was observed for the linkage group with regions restricted to a single arm. For the latter group, as well as one of the three groups encompassing the centromere, the frequency of two-strand and four-strand double crossovers was greater than expected on the basis of a 1:2:1 distribution of two- three-, and four-strand multiple exchanges.

An analysis of five hybrids heterozygous for centromere markers on as many as nine chromosomes revealed only one exception to the independent assortment of the chromosomes. In this one hybrid, a single pair of non-homologous centromeres segregated preferentially in the parental combination.

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EFFECTS OF 5-BROMODESOXYURIDINE UPON GENE RECOMBINATION IN ESCHERICHIA COLI K-12[1]

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GENETIC analyses of crosses of *E. coli* K-12 Hfr × F— have demonstrated that factors possessed by the donor parent (Hfr) are transferred in a characteristic, oriented, and progressive manner to the recipient (F—) parent (WOLLMAN, JACOB, and HAYES 1956), and that recombinants segregate only from the F— parent (LEDERBERG 1956). The transfer of radiophosphorous-containing material (probably DNA) has been correlated with the transfer of genetic factors by GAREN and SKAAR (1958), while experiments of JACOB and WOLLMAN (1954) and PARDEE, JACOB and WOLLMAN (1958) indicate that little, if any, cytoplasmic transfer occurs during conjugation. It is for these reasons that *E. coli* K-12 has proven to be a particularly suitable system in which to test the effects of agents, such as ultraviolet irradiation or radiophosphorous disintegration, upon genetic recombination (JACOB and WOLLMAN 1958). If the donor of genetic material is treated with these agents and permitted to conjugate with appropriate F— cells, nonspecific physiological effects can be resolved from those which affect the genetic material.

By subjecting donor bacteria to low doses of ultraviolet irradiation, significant increases in the frequency of gene recombination have been obtained by JACOB and WOLLMAN (1958). These authors assert that the mechanism best explaining their data is one based upon the partial replica hypothesis (LEVINTHAL 1954). According to this hypothesis, a new recombinant segment of genetic material arises by alternately copying from one, then the other, of two paired homologous strands. They suggest that lesions are formed upon ultraviolet treated genetic material, which represent sites along its length and interfere with normal replication by causing the newly formed strand to switch back to the nonirradiated segment for completion of replication.

Since the mode of action of ultraviolet irradiation is incompletely understood, these experiments do not test directly the partial replica hypothesis. Other agents whose action upon genetic material can be more clearly inferred are desirable to test this hypothesis.

The nucleic acid base analogue 5-bromouracil is specifically incorporated into

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the DNA of *E. coli* B and quantitatively replaces thymine (DUNN and SMITH 1954; ZAMENHOF and GRIBOFF 1954). This analogue, administered as the free base or its desoxyriboside, can act as an inducer of specific mutations for phage T4B (BENZER and FREESE 1958) and is mutagenic for phage T2 (LITMAN and PARDEE 1956) and for thymine requiring strains of *E. coli* (ZAMENHOF, DE-GIOVANNI and GREER 1958). In light of these observations, studies of the effects of 5-bromouracil DNA upon genetic recombination were initiated. It is the purpose of this paper to describe the results of genetic experiments upon *E. coli* K-12 Hfr × F − crosses in which the donor bacteria were grown under conditions of thymine starvation favoring incorporation of the analogue.

MATERIALS AND METHODS

Strains of *E. coli* K-12 HfrC and P678 were obtained from F. JACOB. HfrC bears the markers *thr*$^+$ *leu*$^+$ *azi-s T1-s lac*$^+$ *gal*$^+$ *λs str-s* and is a derivative of HfrH of HAYES (1957). P678 bears the markers *thr leu azi-r T1-r lac- gal- λ-s str-r xyl- mtol-* (ability to synthesize threonine *thr*, leucine *leu*, resistance (*r*) or sensitivity (*s*) to sodium azide *azi*, streptomycin *str*, bacteriophage *T1* or λ; fermentation of lactose *lac*, galactose *gal*, xylose *xyl*, or mannitol *mtol*). Strain P678 was treated with phage T6 and a T6 resistant mutant recovered for use in kinetic experiments. The order of transfer of markers from donor HfrC to recipient P678 is *thr*$^+$ *leu*$^+$ *azi-s T1-s lac*$^+$ *gal*$^+$ with *thr*$^+$ *leu*$^+$ at the origin (WOLLMAN *et al.* 1956). Streptomycin sensitivity and markers concerned with fermentation of xylose and mannitol are transferred with low frequency.

Nutrient agar slants: containing per liter, 8 gm DIFCO dehydrated nutrient broth, 5 gm NaCl, and 20 gm agar. *M-26 basal minimal medium:* (after W. R. SISTROM) contained per liter, K_2HPO_4 8.7 gm, KH_2PO_4 6.8 gm, NH_4Cl 1.0 gm, Na_2SO_4 0.1 gm, $MgCl_2 \cdot 6H_2O$ 0.16 gm, $CaCl_2 \cdot 2H_2O$ 0.002 gm, $FeCl_3 \cdot 6H_2O$ 0.002 gm, glucose 4.0 gm, and thiamin 0.001 gm. *Sulfanilamide medium:* (after BENZER and FREESE 1958) consisted of M-26 plus, per liter, Ca-pantothenate 1 mg, pyridoxine 1 mg, thiamin 1 mg, xanthine 25 mg, hypoxanthine 25 mg, vitamin-free casein hydrolysate 1.0 gm, sulfanilamide 2.0 gm, and uracil 1 mg. *Selective and scoring media:* M-26 1.5 percent agar plates containing 160 mg per liter streptomycin (MS) were used to select *thr*+ *leu*$^+$ *str-r* recombinants. M-26 1.5 percent agar plates with 4 gm per liter galactose replacing the glucose, 80 mg per liter L-threonine, 40 mg per liter L-leucine, and 160 mg per liter streptomycin (MGTLS) were used to select *gal*$^+$ *str-r* recombinants; the same medium without threonine and leucine (MGS) was used to select *thr*$^+$ *leu*$^+$ *gal*$^+$ *str-r* recombinants. Nutrient agar plates containing 100 mg per liter sodium azide or about 10^9 particles of phage T1 were employed to score for azide and T1 sensitivity or resistance, respectively. Fermentation abilities were scored using EMB complete medium supplemented either with lactose or galactose (LEDERBERG 1947).

Vitamin-free casein hydrolysate was obtained from Nutritional Biochemicals Co., streptomycin from C. Pfizer and Co., and 5-bromodesoxyuridine from the California Foundation for Biochemical Research.

Crosses of HfrC × P678 were standardized according to the following protocol. Dilute inocula from nutrient agar slants were spread upon plates of M-26 supplemented with threonine and leucine, incubated at 37°C overnight, and stored at 10°C. From these plates a single colony of each parent was suspended in 20 ml liquid M-26 (plus threonine and leucine for P678) and incubated overnight. Twenty ml amounts of sulfanilamide medium were inoculated with 0.2 ml of the overnight Hfr culture and incubated ten to 11 hours at 37°C without aeration. One portion of sulfanilamide medium contained 50 μgm per ml thymine (control) while others contained various concentrations of 5-bromodesoxyuridine (BD). Simultaneously with the inoculation of the sulfanilamide medium, 20 ml portions of M-26 supplemented with threonine and leucine were inoculated in a similar manner with P678 F— and incubated as above. A sample of each parent was then spread upon each type of selective medium (about 10^7 cells per plate). Both parents were centrifuged separately, washed three times with glucose-free M-26, and resuspended to a concentration of about 2–5 × 10^8 cells per ml. Total cell counts were made with a Petroff-Hausser counting chamber under dark field illumination at 800×. To determine colony-forming ability, plate counts of the HfrC parent were prepared by spreading appropriate dilutions onto M-26 agar plates. The bacteria were mixed in the proportions of 1–2 × 10^7 HfrC per ml to 2–5 × 10^8 P678 per ml. A small amount of nutrient broth concentrate and glucose were added to a final concentration of 0.1 and 0.01 percent, respectively. Five ml of the mating mixture was pipetted into a prewarmed 125 ml flask and incubated on a slow speed shaking water bath (70, ¾ inch strokes per minute) at 37°C. Sixty minutes after mixing, samples of the mating mixture were withdrawn, diluted, and plated on the selective media (MS for *thr*$^+$ *leu*$^+$ *str-r*, MGTLS for *gal*$^+$ *str-r*, and MGS for *thr*$^+$ *leu*$^+$ *gal*$^+$ *str-r* colonies). Recombinant colonies were counted 36 hours after plating. To remove background contamination and to aid in scoring, a sample of 100 to 200 colonies from MGS and MGTLS was spotted onto identical media. The replicas were incubated 12 hours, then replica plated onto suitable scoring media.

The number of recombinant colonies per 100 initial HfrC cells, as determined by total cell counts, will be termed the *recombination frequency*, while the number of recombinants of a given selected genotype bearing a designated HfrC marker among 100 recombinants of this type will be termed the *genetic constitution* of the recombinants (after WOLLMAN *et al.* 1956).

Total cell counts, rather than plate counts, were employed in all determinations of recombination frequency, since the viability of BD-treated cells was variable and low. For some crosses, the frequency of *thr*$^+$ *leu*$^+$ *str-r* colonies recovered exceeded the plate counts of the treated HfrC parent, indicating that cells which no longer can form colonies on M-26 agar plates can donate their genetic material.

To determine the extent of replacement of thymine by 5-bromouracil, DNA from analogue-treated donor cells (60 μgm BD per ml) was isolated by the method of HOTCHKISS (1957), hydrolyzed one hour in 12 *N* perchloric acid at 100°C (MARSHAK and VOGEL 1951), and the resulting bases were chromato-

grammed in the solvent of MacNutt (1952) which permitted simultaneous resolution of adenine, guanine, cytosine, thymine and 5-bromouracil. The bases were located under ultraviolet illumination, cut out, eluted in 0.1 *N* HCl and their amounts determined by the maximal extinction technique of Dunn and Smith (1957). The average replacement of thymine by 5-bromouracil for two samples of DNA, each analyzed in triplicate, was 49 percent. Thus, incorporation of BD into HfrC DNA is extensive under the conditions of growth reported here.

RESULTS

Genetic constitution of gal$^+$ str-r *colonies:* When colonies bearing proximal markers, such as *thr*$^+$ *leu*$^+$ (*str-r*), are selected from the mating mixture, genetic material consisting of segments of various sizes will be transferred to the recipient bacteria. Some of these segments will bear all of the more distal markers such as *lac*$^+$ and *gal*$^+$, while others will not. This effect may be normalized by employing selection for distal markers and examining the inheritance of markers proximal to this. When selection for *gal*$^+$ *str-r* colonies is employed, the entire segment from *gal*$^+$ to the origin must have been transferred, and only effects upon integration will be manifest. The genetic constitution of *gal*$^+$ *str-r* recombinants (Table 1) shows a decrease in the frequency of markers integrated from BD-treated donors. This result can best be accounted for by assuming that portions of the HfrC genetic material which are *integrated* into the zygote are decreased.

This assumption is verified by a consideration of the percent of *gal*$^+$ *str-r* colonies which bear all four of the HfrC unselected markers (Table 2). Here, a striking decrease of this type of colony is evident among matings involving BD-treated donors.

Recovery of recombinant colonies: When the HfrC parent was grown in thymine (control) or various concentrations of BD, comparison of the number of *thr*$^+$ *leu*$^+$ *str-r*, *gal*$^+$ *str-r*, or *thr*$^+$ *leu*$^+$ *gal*$^+$ *str-r* colonies per 100 initial HfrC

TABLE 1

Genetic constitution of gal$^+$ str-r *colonies; from each mating 180 to 250 colonies were examined*

Exp. no.	Treatment of HfrC	Percent HfrC markers integrated			
		thr$^+$ *leu*$^+$	*azi-s*	*T1-s*	*lac*$^+$
I	Control	83	88	63	69
	12μ gm/ml BD	71	85	38	58
	60μ gm/ml BD	58	69	44	46
	120μ gm/ml BD	63	70	37	51
II	Control	69	58	53	64
	12μ gm/ml BD	71	55	51	58
	24μ gm/ml BD	55	47	48	49
	60μ gm/ml BD	58	44	47	56
III	Control	80	59	57	70
	60μ gm/ml BD	66	35	40	49

TABLE 2

Frequency of gal+ str-r *colonies bearing all four HfrC unselected markers*

Exp. no.	Treatment of HfrC	Percent *gal+ str-r* colonies bearing all four unselected HfrC markers
	Control	49.5
	12μ gm/ml BD	25.0
I	60μ gm/ml BD	19.9
	120μ gm/ml BD	27.7
	Control	44.4
	12μ gm/ml BD	33.2
II	24μ gm/ml BD	29.6
	60μ gm/ml BD	30.5
	Control	40.0
III	60μ gm/ml BD	16.0

cells yields the relationship between the dose of BD and the recombination frequency shown in Table 4. The marked decrease in the frequency of all three classes of recombinant colonies can be related to the concentration of BD. The decrease of *thr+ leu+ str-r* colonies is similar to that of *gal+ str-r* colonies and suggests that transfer is not the stage affected. The *thr+ leu+* markers of the HfrC donor are closely linked and penetrate the recipient cell at about eight minutes from the time of mixing the two parents and are, therefore, proximal to the HfrC marker *gal+*, which penetrates the F− cell at about 25 minutes (WOLLMAN *et al.* 1956). A greater decrease of distal to proximal markers would be expected if transfer were the affected stage. The decrease of proximal markers is nearly equal to that of distal markers, clearly indicating that these results are not due to interference with transfer of genetic material but to some effect upon its integration into the zygote or upon the ability of HfrC to form effective contacts. The greater decrease of *thr+ leu+ gal+ str-r* recombinants relative to either *thr+ leu+str-r* or *gal+ str-r* indicates that one effect of BD is upon integration since the simultaneous integration of two widely separated markers is reduced more than that of either factor alone.

A second, lethal, effect of BD is also manifest when the frequencies of recombinant colonies issuing from matings in which the donor was BD-treated are compared to the control values. This is, that the decrease is of a greater magnitude than is the effect upon the integration of markers. For instance, in Experiment II (Table 4) the *thr+ leu+ str-r* colony frequency is decreased by a factor of about six, still a consideration of the percent of nonselected markers integrated (Experiment II, Tables 1 and 3) shows a decrease by a factor of about 0.20. Thus, many of the analogue treated cells are dead in the sense that they no longer can transfer their genetic material or form effective contacts.

Genetic constitution of thr+ leu+ str-r *colonies:* Table 3 presents the genetic constitution with respect to the nonselected markers of *thr+ leu+ str-r* colonies formed from BD-treated and control donor bacteria. A decrease in the frequency of factors integrated from BD-treated donor bacteria is apparent in each in-

TABLE 3

Genetic constitution of thr⁺ leu⁺ str-r *colonies; 180 to 250 colonies from each mating were examined. The HfrC of Exp. IV was a* lac⁻ *derivative of HfrC obtained by ultraviolet irradiation*

Exp. no.	Treatment of HfrC	Percent HfrC markers integrated			
		azi-s	*T1-s*	*lac⁺*	*gal⁺*
	Control	90	73	43	27
	12μ gm/ml BD	80	65	44	25
II	24μ gm/ml BD	78	62	26	14
	60μ gm/ml BD	78	60	32	18
	Control	86	65	41	26
III	60μ gm/ml BD	76	51	19	16
	Control	91	70	..	20
IV	60μ gm/ml BD	74	54	..	6

TABLE 4

The effect of various concentrations of BD upon the recovery of recombinant colonies. For each value 400 to 1,500 colonies were counted

Exp. no.	Dose of BD μgm/ml	*thr⁺ leu⁺ str-r* per 100 Hfr	*gal⁺ str-r* per 100 Hfr	*thr⁺ leu⁺ gal⁺ str-r* per 100 Hfr	a*	b†‡	Percent viable cells
	Control	13.20	3.74	...	3.53	...	98
	12	8.50	2.66	...	3.20	...	100
I	60	3.38	0.88	...	3.84	...	57
	120	2.24	0.56	...	4.00	...	49
	Control	3.89	1.32	...	2.95	...	93
	12	1.35	0.46	...	2.94	...	56
II	24	1.13	0.27	...	4.18	...	52
	60	0.75	0.18	...	4.16	...	34
	Control	15.00	3.62	2.95	4.16	5.10	...
	12	1.60	0.46	0.24	3.56	6.67	...
V	60	0.73	0.13	0.08	5.66	9.13	...
	120	0.17	0.03	0.02	5.66	8.50	...

* a—The ratio of *thr⁺ leu⁺ str-r* to *gal⁺ str-r* colonies.
† b—The ratio of *thr⁺ leu⁺ str-r* to *thr⁺ leu⁺ gal⁺ str-r* colonies.

stance. This decrease indicates that the processes of integration or transfer may be affected, however, as the *thr⁺ leu⁺ str-r/gal⁺ str-r* ratio remained unchanged (see Table 4) it is more probable that integration is the affected stage. If, say, the rate of breakage during transfer were to have been increased, this ratio should also increase.

Kinetic experiments: The HfrC donor was grown in sulfanilamide medium containing either thymine or BD, mated to P678 *T6-r*, and aliquots of the mating mixture were periodically removed, treated with T6 (multiplicity of 100) and KCN (2×10^{-3} *M*) in order to stop transfer of genetic material and to destroy the donor parent (HAYES 1957; FISHER 1957). Estimation of the genetic consti-

tution of *thr*+ *leu*+ *str-r* colonies permitted a determination of the times of entry of the various HfrC markers into recipient cells.

The times of entry and plateau levels of the unselected markers are presented in Table 5 and Figures 1 and 2. It is clear that one effect of the analogue is to lengthen slightly the time required for transfer. Since the HfrC parent requires energy for transfer of genetic material (FISHER 1957), the increase in the time required for transfer may be a nonspecific effect due to the analogue upon the donor cells. The lower values for the percent of nonselected markers integrated obtained from the mating of BD-treated cells can be explained by interference with the processes of integration. Were transfer to be affected by the analogue's increasing the rate of breakage, the plateau levels would be decreased; in addi-

TABLE 5

Results of kinetic experiments: For each cross and sample 100 colonies were scored

Treatment of HfrC	*azi-s*	*T1-s*	*lac*+	*gal*+	
50μ gm per	88	66	30	11	plateau levels (in percent)
ml thymine	10	10	25	35	times of entry (in minutes)
60μ gm per	79	39	10	6	plateau levels (in percent)
ml BD	20	25	35	45	times of entry (in minutes)

Samples of the mating mixture were taken at ten minute intervals and treated as described. The plateau levels represent the genetic constitution of the *thr*+ *leu*+ *str-r* colonies and are averages of values obtained from several samples of the mating mixture taken after the markers had reached their maximum frequency of integration. The times of entry are accurate to the nearest five minutes.

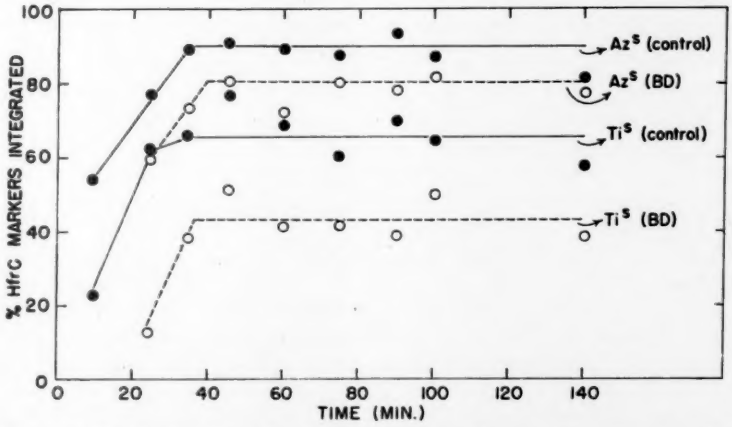

FIGURE 1.—The kinetics of transfer of different markers. The genetic constitution of *thr*+ *leu*+ *str-r* colonies formed at the times indicated has been analyzed, and these values plotted as a function of time. Only the times of appearance of the *azi-s* and *T1-s* markers have been graphed; *lac*+ and *gal*+ have been omitted for clarity (see Figure 2). Solid lines represent control values; dotted lines, values obtained from matings in which the donor was grown with 60 μgm per ml 5-bromodesoxyuridine (see Table 5).

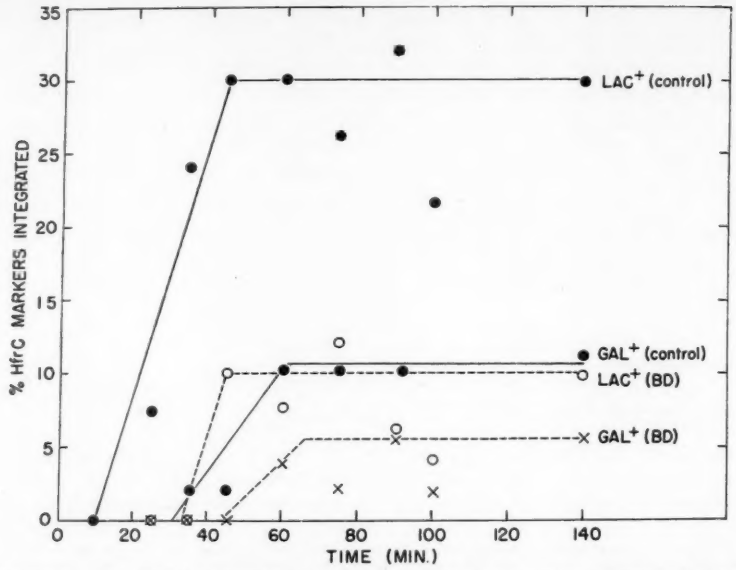

FIGURE 2.—The kinetics of transfer of different markers. The genetic constitution of *thr*$^+$ *leu*$^+$ *str-r* colonies formed at the times indicated has been analyzed, and these values plotted as a function of time. Here, only the times of appearance of the *lac*$^+$ and *gal*$^+$ markers have been graphed; see Figure 1 for *azi-s* and *T1-s*. Solid lines represent control values; dotted lines, values obtained from matings in which the donor was grown with 60 μgm per ml 5-bromodesoxyuridine (see Table 5).

tion, the rate at which a plateau is attained would be decreased, which is not the case. Were integration the affected stage, only the plateau levels would be decreased, which is the observed result. Further proof that integration is affected is evident when transfer effects are ruled out by selection for distal markers and analysis of the unselected markers transferred before the distal markers (see Tables 1 and 2).

Control experiments: The possibility remained that the genetic effects described were due to treatment of the donor with sulfanilamide and directly resulted from conditions of thymine starvation and not from replacement in the DNA of thymine by bromouracil. To test this, HfrC was grown in sulfanilamide medium containing neither BD nor thymine, mated to P678, and the genetic constitution of resultant *thr*$^+$ *leu*$^+$ *str-r* recombinants was examined. No differences were observed between these results and those of concomitant control matings. For effects upon integration to be manifest, BD must at least be present in the sulfanilamide medium. Prolonged conditions of thymine starvation did lead to a marked decline in the frequency of recombination. This decline is due to death of the treated donors and their inability to form effective contacts, or to transfer their genetic material.

The possibility that BD-treated HfrC bacteria underwent mutation at the loci employed for these genetic experiments is remote. To explain the observations on this basis many simultaneous mutations at a number of specific loci must be invoked. Nevertheless, this possibility was tested. BD-treated HfrC bacteria were mated and genetic information obtained (see Experiment III in Tables 1, 2, and 3). A portion of the unmated HfrC bacteria was retained and plated onto all four scoring media at a concentration of about 5×10^7 cells per plate. These plates were incubated and examined the following day. No mutations were observed from the HfrC genotype but for several *T1-r* colonies which were also present when thymine-grown cells were plated.

DISCUSSION

The above results clearly demonstrate that a decrease in the frequency of HfrC markers recovered among recipient cells is due to an effect of the analogue upon some stage of integration of the genetic material. This is evident when a distal marker, *gal*$^+$, is selected and the recovery of markers proximal to it, which must have been transferred, is examined (Table 1). Furthermore, when genotypes of colonies bearing the distal marker are compared, those colonies which were derived from matings of BD-treated donors bearing all four HfrC markers were reduced by a factor of two (Table 2). To explain the decrease in frequency of this kind of *gal*$^+$ colony, the average number of switches between *thr*$^+$ *leu*$^+$ and *gal*$^+$ has to be increased by 0.7 (suggestion of PROFESSOR C. LEVINTHAL). If the frequency of *thr*$^+$ *leu*$^+$ *azi-s T1-s lac*$^+$ *gal*$^+$ *str-r* colonies among all *gal*$^+$ *str-r* colonies selected is symbolized by a, and if one assumes no switches must occur between *thr*$^+$ *leu*$^+$ and *gal*$^+$ to yield this kind of colony, then the average number of switches occurring in that region is n, where:

$$e^{-n} = a$$

For control crosses e^{-n} is 0.49 and n is 0.69, while in BD crosses e^{-n} is 0.25 and n is 1.39. Thus, the average number of switches is increased from 0.69 to 1.39, or by a factor of two.

Other effects of the analogue, too, are present and include death of BD-treated cells in the sense that their ability to transfer genetic material or to form effective contacts is lost. These effects are seen most clearly in the results presented in Table 4 where the ability of BD-treated donors to form recombinants decreases at a greater rate than the loss of colonies bearing simultaneously all HfrC markers (Table 2).

The analogue also affects transfer, increasing the time required for markers to enter the recipient. This effect was resolved from that upon integration as a transfer effect would be negated when distal markers were selected. If a mating between an Hfr donor and F$^-$ recipient bacterium is considered from the viewpoint that the transferred genetic material is rescued and integrated into the F$^-$ genome by recombination, the presence in treated donors of BD appears to result in the integration of smaller regions of the transferred genetic material. This effect can best be interpreted by the partial replica hypothesis of LEVINTHAL (1954) and

JACOB and WOLLMAN (1955, 1958) in which integration of genetic material proceeds by pairing of homologous segments and the formation of a new replica between them. This replica may copy alternate regions of the paired segments by switching from one to the other. When one of the two paired parental segments is altered, in this case by the presence of Hfr bromouracil DNA, copying is forced away more frequently to the other parental (F^-) segment. The presence of the analogue in Hfr DNA appears to act in much the same fashion, but not to the extreme degree as ultraviolet treated genetic material (JACOB and WOLLMAN 1955, 1958; WOLLMAN *et al.* 1956).

Two hypotheses can be presented to account for the observed increase in the frequency of recombinant events. In the first, pairing could be disrupted so that partial replicas can only be formed from the paired regions of Hfr genetic material. The second assumes pairing to remain unaffected and requires that sites along the length of the Hfr genetic material discourage the formation of partial replicas. An experiment to distinguish between these two hypotheses is in progress. If the former hypothesis is true, it is predicted that the frequency of recombinant types from closely linked markers will decrease; if the latter, an increase in the frequency of recombinant types is expected.

Recently, EPHRATI-ELIZUR and ZAMENHOF (1959) observed that transforming ability of bromouracil DNA of *B. subtillus* is present, but the activity is either decreased or unaltered depending on the markers observed. These results are not incompatible with those presented in this paper. Both may be explained by the hypothesis that rescue of markers present on bromouracil DNA occurs by the mechanism of partial replica formation in which the replicas are biased away from the genetic material containing the analogue.

BENDICH, PAHL and BROWN (1957), in studies on the chromatographic fractionation of *E. coli* DNA containing C^{14}-5-bromouracil, have demonstrated that the DNA is heterogenous with respect to the incorporation of 5-bromouracil. Their work raises an important consideration in the interpretation of the results of this paper. Does transferred genetic material actually contain the analogue, or does the bromouracil-containing DNA remain in the donor, and is the effect a more subtle one? From the observed great extent of replacement of thymine by bromouracil it appears unlikely that one third of the genome of HfrC donors can remain free of the analogue; and the transferred genetic material represents one third of the content of a haploid nucleus (STENT and FUERST 1959). The heterogeneity of 5-bromouracil incorporation is most likely due to a populational heterogeneity of the analogue-treated cultures.

SUMMARY

The effects of the thymine analogue 5-bromodesoxyuridine upon genetic recombination in *Escherichia coli* K-12 have been investigated. When Hfr donor bacteria are treated with the analogue under conditions of thymine starvation and mated to untreated recipient bacteria, both the stages of transfer and integration of genetic material are affected by the analogue. The effects of the analogue

upon each stage have been resolved. The average number of switches occurring over a given region is increased by a factor of two. Analysis of the genetic constitution of recombinant progeny suggests that the rescue of factors contained on the transferred genetic material is hindered so that smaller portions are integrated. These results can be best explained by the partial replica hypothesis of LEVINTHAL and JACOB and WOLLMAN.

ACKNOWLEDGMENT

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SELECTION FOR CHOLINESTERASE ACTIVITY IN THE CEREBRAL CORTEX OF THE RAT[1, 2]

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KRECH, ROSENZWEIG, BENNETT, and KRUECKEL (1954) found strain differences in cerebral cortex cholinesterase activity (ChE) of the rat as well as evidence that some behavioral characteristics might be associated with ChE levels. Since then, studies of ChE and adaptive behavior have afforded several inroads into the understanding of the general biological significance of this enzyme (ROSENZWEIG, KRECH and BENNETT 1958a), beyond that already well established from basic neurological studies.

Selection experiments have been concerned with a wide variety of traits in many different organisms. With respect to biochemical studies, WOODWORTH, LENG and JUGENHEIMER (1952) have described a 50 year selection for protein and oil content in corn. A recent example of the several selection programs, in which the selection criterion is high and low response of an animal to an injected agent, is the experiment of KYLE and CHAPMAN (1953) who selected in the rat for high and low response of the ovary to a standard injected dosage of a gonadotrophic hormone. WEIR and CLARK (1955) successfully selected for high and low blood *p*H in the mouse. No attempt has yet been made to modify by selection the activity of an enzyme.

This selection experiment was undertaken as part of an effort to: (1) find or produce other strains of rats with ChE differences for studies on the relationship of ChE and adaptive behavior, and (2) to obtain information on the genetics of modifiers of enzyme activity.

MATERIALS AND METHODS

Two foundation stocks of rats were used, from each of which a high and a low ChE line have been derived. Both stocks, obtained from animals maintained by the Small Animal Breeding Colony of the Genetics Department, University of California, Berkeley, were genetically heterogeneous, although their genetic backgrounds were different. One stock (RC), obtained from PROFESSOR W. E. CASTLE, was a product of crosses of many different strains from various sources used in his linkage studies. The other stock (RD) was descended from a four-way cross of

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[2] From a dissertation submitted in partial fulfillment of the requirements of the degree of Doctor of Philosophy, University of California, Berkeley.

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four inbred lines of different origin. The four original lines were: (1) U.C. Line A (albino) from Iowa State University, (2) U. C. Line I (agouti hooded) from August Line 990, Wayne University, (3) U. C. Line F (albino) from King A Albino Line, Wistar Institute, and (4) U. C. Line J (albino) from Fischer Line 344, Wayne University.

A double first cousin mating system was used in each generation and in each line throughout the experiment. This mating system was chosen because it maintained maximum heterogeneity for the population size adopted. A more open breeding system would have maintained greater genetic heterogeneity but would have necessitated the care and analysis of a cumbersome number of animals.

Reciprocal crosses were made between two litters within each line in each generation (Figure 1). Matings were made randomly between members of the two litters. After the birth of offspring from these matings, the male parents only were sacrificed for chemical analysis in the 200–250 day age range over which ChE activity remains relatively stable (BENNETT, ROSENZWEIG, KRECH, KARLSSON, DYE, and OHLANDER, 1958). The ChE analyses were made, and the offspring from the highest sire (in the case of a high line or the lowest in the case of a low line) in each of the parental litters were saved, thus affording two new litters with which the process was repeated in the next generation. The measurements on which selection was based were, in all cases, deviations from litter means so that possible maternal and other cage effects did not affect selection.

The assay of ChE activity has been reported elsewhere in detail (KRECH, ROSENZWEIG and BENNETT 1956). The animal was decapitated, and samples from the visual (V) and somesthetic (S) areas of the cerebral cortex were excised (Figure 2). The remainder of the cortex was stripped away, leaving the sub-

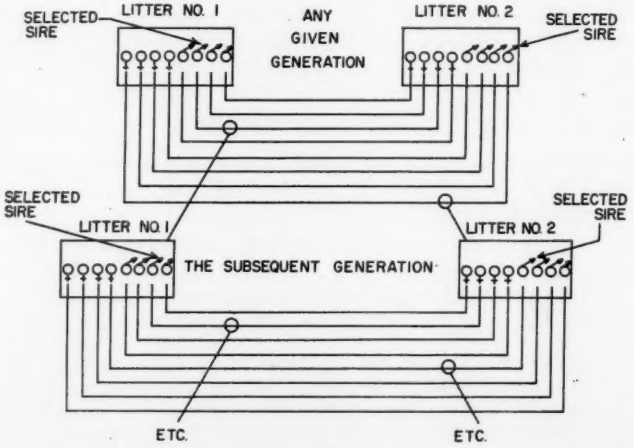

FIGURE 1.—The double first cousin mating scheme.

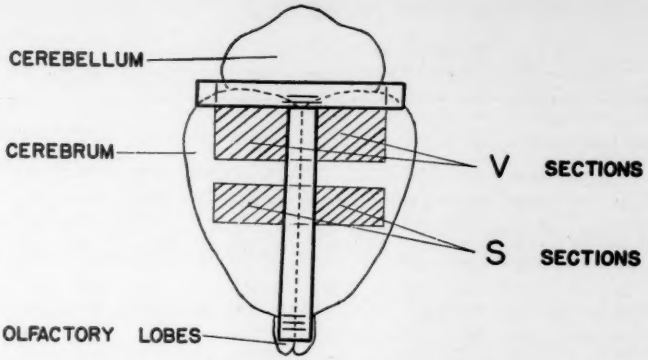

FIGURE 2.—Rat brain and superimposed T square with which the areas to be ablated are delineated.

cortex, olfactory lobes and cerebellum, all of which comprise the subcortical sample. These samples were then weighed rapidly, quick-frozen on dry ice, and stored at −20°C until analysis which followed within the week. Just before the analysis, the sample was homogenized in cold 0.9 percent saline. With the use of a Nielands-Cannon Automatic Titrator (NIELANDS and CANNON 1955), the enzyme activity was determined under standardized conditions by the rate of hydrolysis of acetylcholine perchlorate (ACh). The activity of the sample was expressed as moles of ACh $\times 10^{10}$ hydrolyzed per minute per milligram of tissue. The cortical ChE activity was taken as the average value of the visual and somesthetic areas $[(V+S)/2]$.

RESULTS

The results of selection are given in Table 1 and Figure 3. In Figure 3, each point beyond the foundation stock generally represents the mean of males of two litters taken as a combined array. The standard deviations of the means about each point are also statistics of the male individuals of the two litters grouped together. The environmental effect between generations is large, as indicated by the extent of the vertical zigzagging of the means between generations. Whenever possible, a high line litter and a low line litter were assayed at the same time in an effort to minimize these environmental effects within generations, and therefore to make the high-minus-low value a more efficient statistic. However, it was not usually possible to assay both high litters or both low litters comprising each point at the same time. As a result, some extra day-to-day environmental variation is incorporated into the standard error of each mean. The larger standard errors, from this incorporation, make the high-minus-low values appear less significant than if standard errors depended only on within-litter variation. This alteration is of little importance, as the litter means within each line in each generation vary so little; the within generation variance is obviously negligible compared with the between generation variance

TABLE 1

Results of selection for ChE activity.
Number (N), *mean* ($\bar{x}$), *standard deviation* (s), *standard error* ($s_{\bar{x}}$)

Sel. gen.*	Line	N	$\bar{x}$	s	$s_{\bar{x}}$	High minus low	Line	N	$\bar{x}$	s	$s_{\bar{x}}$	High minus low
S_0	RD	14	59.04	4.53	1.21		RC	14	57.76	6.42	1.05	
S_1	RD high	8	59.65	3.51	1.24	− 1.77	RC high	4	57.65	2.23	1.11	5.56
	RD low	7	61.42	5.41	2.05		RC low	9	52.09	5.38	1.79	
S_2	RD high	9	62.30	3.28	1.09	14.31	RC high	8	60.41	5.63	1.99	3.99
	RD low	8	47.99	3.03	1.07		RC low	6	56.42	5.55	2.27	
S_3	RD high	10	58.77	4.30	1.36	5.52	RC high	9	53.29	2.62	0.87	7.91
	RD low	7	53.25	2.44	0.92		RC low	6	45.38	3.91	1.59	
S_4	RD high	10	63.60	3.45	1.09	12.48	RC high	9	58.81	3.32	1.10	6.95
	RD low	8	51.12	2.27	0.80		RC low	8	51.86	2.71	0.96	
S_5	RD high	11	61.03	4.09	1.23	13.91	RC high	15	54.27	3.31	0.86	9.13
	RD low	8	47.12	2.19	0.77		RC low	13	45.14	2.43	0.68	
S_6	RD high	8	60.67	2.11	0.75	12.04	RC high	5	64.37	2.43	1.09	16.54
	RD low	8	48.63	2.67	0.95		RC low	8	47.83	2.87	1.01	

* Selected generation.

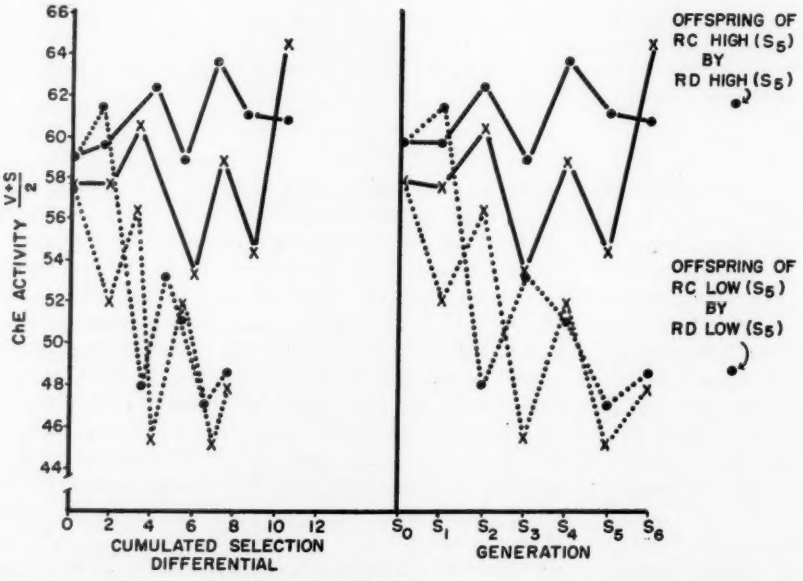

FIGURE 3.—Results of selection for cortical ChE.

as indicated by the values of $s_{\bar{x}}$ compared with the generation shifts within a line.

Selection was successful almost immediately, and the lines remained apart in the succeeding generations. In all the 12 pairs of generation points represented in both stocks, only one pair, RD S_1, is reversed from the direction selected. The probability, that of 12 independent comparisons, 11 or more would by chance be in agreement with the direction of selection, is

$$(\tfrac{1}{2})^{12} + 12(\tfrac{1}{2})^{11}(\tfrac{1}{2}) \text{ or } 13(\tfrac{1}{2})^{12} = 0.0032.$$

There were no overlappings of the high and low distributions for generations five and six for the RC lines and generations two, four, five and six for the RD lines.

To ascertain whether selection continued to separate the high and low lines as the experiment progressed, regression lines of the high-low difference for both stocks were estimated, each generation contributing one point to the array to which the lines were fitted (Figure 4). Also, a regression line was fitted to an array of points based on the average of the divergence of both stocks for each generation. An analysis of variance was performed to test the significance of these regression lines.

$P = 0.18$ (regression line of separation of RD lines).
$P = 0.02$ (regression line of separation of RC lines).
$P < 0.01$ (regression line of average of separation of RD and RC lines).

From these data it is observed that selection was successful in separating the lines by approximately two ChE units per generation. The direction and extent of the deviations from regression in the latter generations do not give any indication of an incipient plateau.

The significant positive linear regression of the separation of lines is evidence that selection acted upon a polygenic system. Crosses were made between the S_5 generations of the two stocks so as to form a high-by-high line and a low-by-low line. The mean ChE values of the offspring of each cross are comparable in magnitude to the means of the parental stocks for the S_6 generation (Figure 3). These results indicate that even stocks of very different ancestry do not differ with respect to genes showing dominance for ChE activity, or if they do, that the direction of dominance at different loci is almost exactly balanced in plus and minus directions in both high or both low lines. The latter alternative would be exceedingly unlikely. Epistatic effects of gene differences between the stocks, if present, would be expected to result in shifts of the ChE values of the progeny. If different epistatic effects existed between the two stocks, the means of the offspring between the stocks would be expected to shift from their midparent values. Unless identical genes were selected in the two unrelated foundation stocks, it can be concluded that the high and low lines differ mainly in genes that are largely additive.

Heritability: The gain over reach, or realized heritability (h^2), was calculated as follows: The value of the reach for each selected sire was taken as his deviation

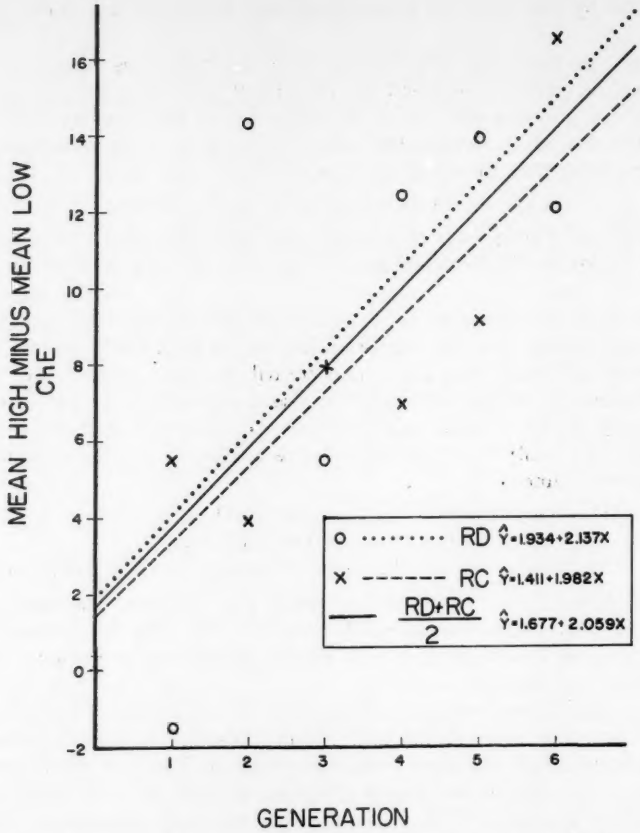

FIGURE 4.—The regression of mean high ChE minus mean low ChE on generation.

from his own litter mean. These values were averaged for the selected sires of the two litters of each line within each generation and then divided by two, because only sires were selected. The gain was estimated as the average value of the unweighted litter means of the succeeding generation, minus the average value of the sires' unweighted litter means. The h^2 estimates (Table 2), therefore, are within-litter estimates where the gain is approximately some constant times the additive genetic variance within litters. The reach is the same constant times the total variance within litters and would not include any variance from maternal or other litter effects.

Although the separations of high and low selected lines give adequate estimates of the average reaction of each strain to plus and minus selection, the reason for the apparent extreme response in both low lines remains an enigma.

TABLE 2

Realized heritability (sum gains/sum reaches)

Stock	High	Low	High and low combined	All lines combined
RD	0.13	1.39	0.67	
				0.78
RC	0.58	1.31	0.89	
Estimates when gain is based on regression line				
RD			0.80	
				0.78
RC			0.74	

It is possible that the whole assay technique has for some reason gradually yielded lower ChE values, although assays of other strains, for which the mean values of ChE are well established, have shown no downward trends. Inbreeding depression is not a factor because the high-by-high and low-by-low crosses, with inbreeding coefficients of zero, fall right in place with the S_6 generation of the original selected lines.

The numerators of h^2 computed by gain over reach depend only on the means of the litters of the first and final generations. To reduce this possible sampling error and to give equal weight to the gains in intermediate generations, total gain was also computed from the least squares regression line of Figure 4. It is, to some extent, coincidental that both estimates for all lines combined are exactly the same. It is interesting to note that the selection differentials were almost identical between the two high lines and between the two low lines. Wherever possible the extreme animals were selected, and no attempt was made to control the differentials in any way.

At the outset of the program, heritabilities were estimated in two other ways. An estimate of 0.163 with 95 percent confidence limits of 0.004 and 0.692 was made from a comparison of five inbred strains. This estimate is based on the assumptions that the strains are completely homozygous, and that they are unrelated strains picked at random from a hypothetical population of strains. Since these assumptions are not completely valid, the estimate is probably an underestimate of h^2. The other estimate was obtained by comparing phenotypic and genotypic relationships between sibs of the foundation stocks. The RD h^2 estiestimate was 0.069 with 95 percent confidence limits of −.570 and 1.559. The RC h^2 estimate was 1.423 with 95 percent confidence limits of 0.525 and 1.947. These heritabilities are estimated assuming that the genetic variance between sibs is half that of the whole population. If this assumption is not completely true (and there is doubt about it in the RD stock, as it was derived from a cross between two lines descended from an original four-way cross), then the estimate would tend to be biased in the minus direction. Also, the assumption is made that besides common genetic effects, no common litter effects, such as maternal effects, prevail; such effects would produce a bias in the plus direction.

Natural selection: That success in the high direction was markedly less than

in the low direction suggests that natural selection may have opposed upward selection. The high lines were in one respect characteristic of animals with lowered fitness if body weight in this case can be considered a manifestation of fitness. Figure 5 summarizes the values of the mean body weights at the time of ChE determination for each line for the S_5 and S_6 generations plus the S_1 generation of the high-by-high and low-by-low crosses. Earlier generations were not weighed. Again, as in the ChE summary, the two or three litters making up each point are taken as single arrays in computing their standard errors. Visual comparison of the data of the high and low lines both between and within stocks, and between the crosses reveals an obvious negative association between litter mean cortical

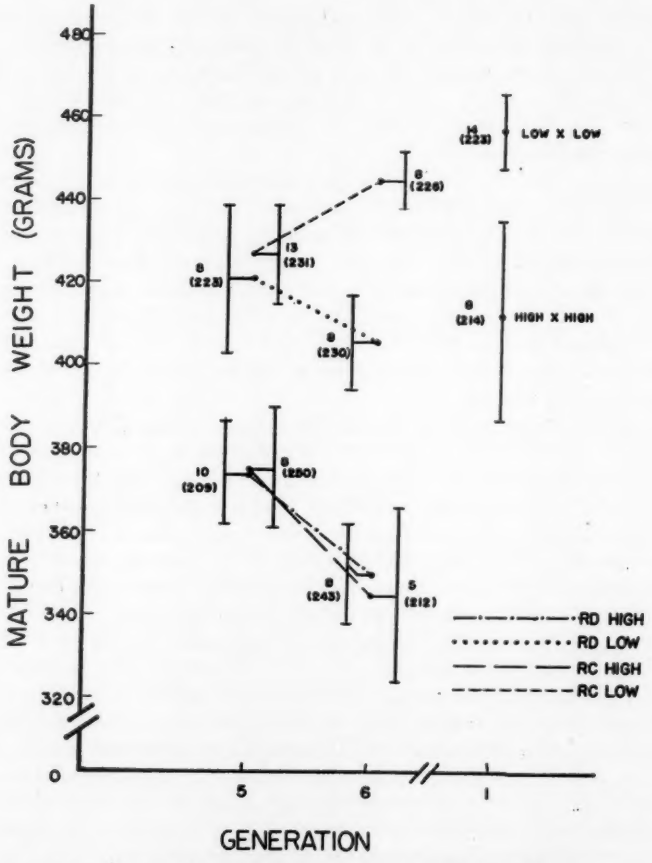

FIGURE 5.—Mature body weight with N, $s_{\bar{x}}$, and average age in parentheses.

ChE and litter mean body weight. Although heterosis appears to be manifested in the body weight means of both cross lines, the separation remains.

Disregarding the means and taking only *within-litter* correlations between ChE and body weight (i.e., a value of each variate is the deviation from the litter mean) of all animals of generations S_5 and S_6 and the S_1 of the cross lines, led to the following result: within-litter correlation of ChE and body weight $= -0.19$; $N = 97$; $P < .05$ (two-tail test). The negative relationship thus holds both between the lines and within the litters. That the within-litter correlation is in part due to a genetic correlation is evidenced by the separation of the body weight means through selection for ChE.

There are now some preliminary behavioral data on the selected lines (KRECH, personal communication) which seem to indicate that the high ChE lines are poorer than the low lines in adaptive behavior as measured by a variety of maze situations. Adaptive behavior may be considered a manifestation of fitness.

Reproductive fitness in terms of percent sterility did not differ between the high and low lines. Also mean litter size was apparently not affected by selection.

Information from variables concomitant to selection concerning the mechanism of selection: It is important to understand the physiological mechanisms through which selection has acted to alter the phenotypic expression of ChE activity if the implications of enzyme activity alteration by genetic means are to be considered. In these experiments, because activity is measured per unit weight of tissue, it is necessary to know, for example, if there are merely more cells per unit weight in high ChE animals than in low ChE animals (with the assumption that more cells mean more nucleoprotein and therefore more enzyme ChE). For the psychologist interested in ChE activity and behavior, important generalizations from psychological studies on these lines might be based on the hypothesis that the mode of action of selection has been primarily through some direct biochemical alteration of ChE activity. If ChE activity had been altered because of gross neurological tissue changes, then it would be more reasonable to study gross tissue changes and behavior, with ChE activity as an interesting side light. It is desirable to distinguish between such mechanisms as this and the more direct mechanisms probably of an intracellular biochemical nature.

During the selection program other variables than ChE activity were measured to find out if any correlated responses to selection did occur. From these observations an attempt has been made to begin the clarification of the mode or modes of the physiological action of selection.

Brain size: With the use of vernier calipers, lengths and widths of the cerebrum were measured for all assayed animals beginning with generation S_2 (Figure 6). The probability that chance alone could have permitted the consistant difference observed in lengths for all generations measured is:

$$2\,[(\tfrac{1}{2})^{11} + 11(\tfrac{1}{2})^{10}(\tfrac{1}{2})] = 0.017 \text{ (two-tail test)}$$

for widths:

$$2\,[(\tfrac{1}{2})^{11} + 11(\tfrac{1}{2})^{10}(\tfrac{1}{2}) + 55(\tfrac{1}{2})^{9}(\tfrac{1}{2})^{2}] = 0.065.$$

The length and width differences, which are in the same direction, when con-

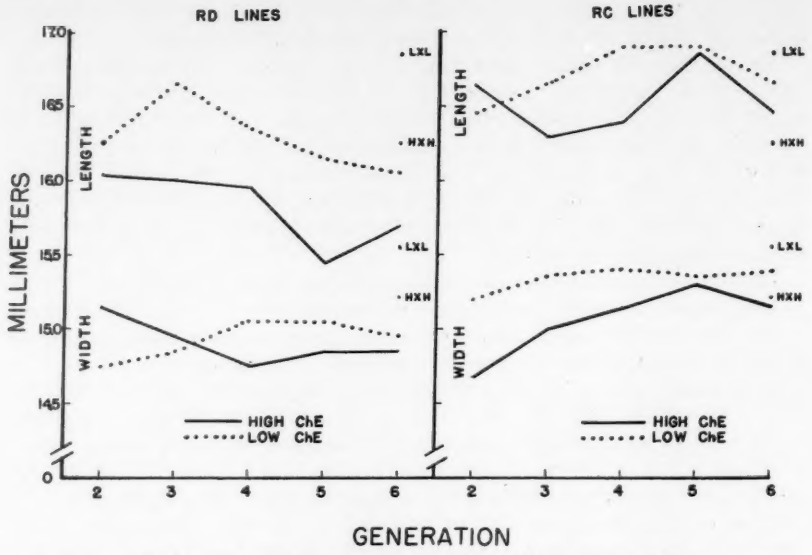

FIGURE 6.—The change in cerebrum length and width with selection for ChE.

sidered together indicate that the smaller brain dimensions of the high ChE lines is highly significant.

ROSENZWEIG, KRECH and BENNETT (1958a) found a gradient of ChE activity over the cerebral cortex of the rat; ChE decreases from anterior to posterior. Thus, the somesthetic area of the cortex has a higher activity than the visual area. The *V* and *S* areas of the cortex (before they were ablated for assay) were carefully delineated on the exposed brain by the use of a small plastic T square (Figure 2). The T square was centered on the cortex and the position of the *S* section was determined by delineating a specific area at the center of the cortex. The position of the *V* section was then determined by measuring a definite distance posterior to the *S* section. Because the *S* areas were taken from the center of the cortex, it is reasonable to consider them physiologically comparable between the high and low lines if selection has not affected the gradient in some fashion. The *V* sections might, however, for reasons of differences in brain length, be histologically different between high and low animals, since the *V* sections were determined by marking a fixed distance from the *S* section. However, since the ChE values of the *V* sections remained approximately 6.3 ChE units below the *S* sections in both high and low lines throughout the program, there is no evidence that possible sampling of histologically different areas is either assisting or counteracting ChE separation between high and low lines.

Brain specific gravities: Since brain dimensions were smaller in the high lines, it was thought possible that brain densities might also have changed with selec-

tion and that a study of any differences in brain density might shed further light on the physical basis for ChE separation brought about by selection. Brain specific gravities were measured from animals of the S_6 and S_7 generations of the four original lines plus the S_1 of the cross lines (Table 3). Since immersion of the brains in water for the determination would have altered the weights of the cortex in any subsequent ChE determination of the same brains, the regular ChE assayed males could not be used. Instead, females sibs of the males were used. The females were sacrificed between 200 and 300 days of age, their brains removed and weighed in air, and then weighed in distilled water. The brain weight in air divided by the loss of weight in water (corrected for temperature variation of the water) was taken as the specific gravity of the brain. A test of the difference between the means of the high and low line shows that the animals from the high ChE lines have a significantly greater brain density than do animals from the low ChE lines:

$$F^{1}_{36} = \frac{6917.0}{852.2} = 8.12,$$

$$P < 0.01.$$

The interaction and between stock variations were not significant.

This association, of course, does not help to explain the ChE separation from selection, as ChE is measured per unit weight tissue; however, the high lines would show even higher ChE if it were determined per unit volume instead of weight.

Since protein is denser than water and most other major cell constituents, an important question is whether the protein percentage has merely increased in the brain cells of the high line animals and decreased in the low, to give the observed ChE separation. An investigation of protein percentage by weight in the cortical and subcortical brain tissues has been undertaken (E. L. BENNETT, personal communication), and the preliminary results do not suggest any association with ChE.

Subcortical ChE: On some of the animals from the S_5 and S_6 of the original and the S_1 of the cross lines, subcortical ChE values were determined. Subcortex here means the whole brain minus the cerebral cortex which was stripped off. The separation of subcortical ChE between the lines was highly significant (Table 4). The within-litter coefficients of variation for cortical ChE and subcortical ChE were 4.79 percent and 3.26 percent, respectively.

TABLE 3

Means of brain specific gravity

Stock	Selected high ChE lines		Selected low ChE lines	
	$\bar{x}$	N	$\bar{x}$	N
RD	1.0405	7	1.0390	7
RC	1.0396	7	1.0350	7
Cross lines	1.0395	7	1.0378	7

TABLE 4

A comparison of cortical ChE and subcortical ChE of the same animals with respect to their separations between high and low lines selected for cortical ChE

		Cortex					
Stock	Generation	High $\bar{x}_1$	Low $\bar{x}_2$	$\bar{x}_1-\bar{x}_2$	t	df	P
RD	S_5	57.11	44.96	12.15	12.15	9	<.0005
	S_6	60.68	48.63	12.05	20.00	14	<.0005
RC	S_5	53.40	44.59	8.81	11.17	15	<.0005
	S_6	64.37	47.83	16.54	21.94	11	<.0005
Cross lines	S_1	61.46	48.48	12.98	18.88	16	<.0005

		Subcortex					
Stock	Generation	High $\bar{x}_1$	Low $\bar{x}_2$	$\bar{x}_1-\bar{x}_2$	t	df	P
RD	S_5	128.30	122.50	5.80	3.09	9	<.01
	S_6	134.25	125.88	8.37	6.27	14	<.0005
RC	S_5	130.88	130.33	0.55	0.19	15	>.25
	S_6	152.20	122.75	29.45	9.67	11	<.0005
Cross lines	S_1	132.75	125.79	6.96	6.85	16	<.0005

TABLE 5

Summary of correlations (r) *by lines between cortical and subcortical ChE*

Line	*r*	*N*
RD high	+ .96	11
RD low	+ .48	16
RD lines combined	+ .56	17
RC high	+ .73	13
RC low	− .04	17
RC lines combined	+ .50	30
High-by-high	+ .71	4
Low-by low	+ .37	14

The correlation between cortical and subcortical values was estimated in a number of ways:

	r	*N*
over-all	+.68;	75
within litters	+.50;	75
between litter means	+.77;	20
between line means, generations separate	+.82;	6

All are significant at the one percent level, although none are independent. These correlations are broken up into correlations within individual lines in Table 5.

Although there do not appear to be any differences in the magnitude of these correlations between the stocks, there is some indication that the high selected lines have a higher correlation. (The insignificantly negative RC low correlation may have resulted from sampling error. Although the difference between the RC high correlation and the RC low correlation is significant at the one percent level, more data on the RC animals would be helpful to determine whether the magnitude of the difference is as great as observed in these data.) However, ROSENZWEIG, KRECH, and BENNETT (1958b) found lower correlations in higher ChE lines in two comparisons, one of which involved other animals from the RC stock. The differences observed here may be fortuitous.

The conclusion to be drawn from these data is that a considerable positive correlation exists between cortical and subcortical ChE. The author feels that the same general conclusion can be drawn from the data of ROSENZWEIG, KRECH, and BENNETT (1958b). The fact that their correlations are not within-litter correlations may account for the general difference in magnitude between their correlations and those reported here.

Thus, the estimate of the correlation between lines is similar to the correlation estimate within litters. Their similarity in magnitude suggests they are estimates of the same value and is evidence for a large genetic component of the correlation.

An important consideration is whether cortical ChE as measured by $(V + S)/2$ is as highly correlated with subcortical ChE as it is with ChE of other areas of the cortex. The motor sections (sections of the cortex comparable to the S section in size but more anterior in location) of 16 animals of the cross lines were assayed. These animals comprised two high and two low line litters of which the mean values were:

Line	Litter	Litter mean ChE (M section)
H × H	514a	70.0
	515	71.8
L × L	618	58.7
	615a	58.5

The within-litter correlation between $(V + S)/2$ and M of all 16 animals was .74 which is significant at the one percent level. The similarity of magnitude of the correlations suggests that selection for ChE in an area of the cortex will affect the ChE in the unselected subcortex almost as greatly as ChE in any other unselected area of the cortex.

Brain weight: Although the brain densities are greater in the high lines, the brain weights are lower. Figure 7 shows the means of the brain weight for the S_5 and S_6 generations of the original lines and the S_1 of the cross lines. The negative association between these two variables holds in general, but is more obvious between the lines within each stock and between the cross lines. If body weight and brain weight were correlated, it would not be surprising to find the ChE-brain weight association since, as already discussed, a negative correlation exists between body weight and ChE. Comparing the mean body and mean

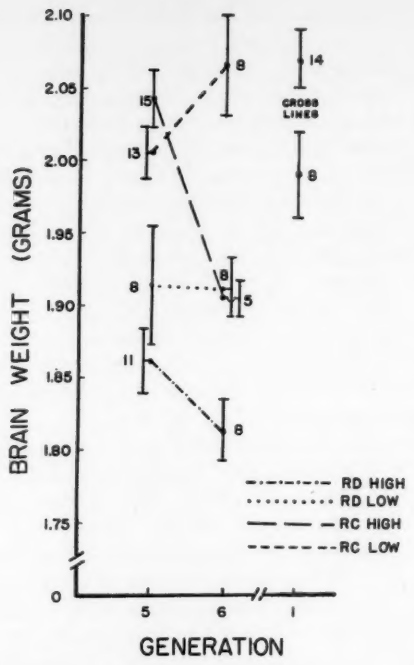

FIGURE 7.—Mean brain weights of each line with N and $S_{\bar{x}}$.

brain weights of these animals, a correlation of +.83 ($P < .01$) was found.

Correlations between these three variables were also estimated within the litters, i.e., taking the value of each variate of each animal as its deviation from its own litter mean. The results based on 97 animals are summarized here with the one-tail probability of each correlation.

ChE, brain weight	$r = -.22$; $P = .010$
ChE, body weight	$r = -.19$; $P = .025$
body weight, brain weight	$r = +.33$; $P < .001$

These three correlations were evaluated for each of the two stocks and the cross lines, respectively (Table 6). Also, from 75 of the 97 animals used in estimating these correlations, data of the subcortical ChE were obtained as already presented. In Table 6 are also the correlations between subcortical ChE and brain weight, and subcortical ChE and body weight. The within-stock correlations between the ChE variables and the weight variables in general support in direction the total correlations. KRECH, ROSENZWEIG and BENNETT (1958) found a strain difference in the magnitude of the correlation between subcortical ChE and brain weight. More data on the individual stocks would be necessary to determine if

TABLE 6

Within-litter correlations and partial correlations for the variables: cortical ChE, subcortical ChE, brain weight, and body weight where w *is subcortical ChE,* x *is cortical ChE,* y *is brain weight,* z *is body weight*

	RD stock	RC stock	Cross lines	Total
N	27	30	18	75
r_{wy}	− .64***	− .32*	− .20	− .40***
r_{wz}	− .44**	− .26	− .12	− .31***
r_{yz}	+ .54***	+ .16	+ .52**	+ .38***
$r_{wy.z}$	− .53***	− .29*	− .16	− .33***
$r_{wz.y}$	− .15	− .22	− .02	− .18
N	34	41	22	97
r_{xy}	− .26	− .17	− .26	− .22*
r_{xz}	− .13	− .25*	− .19	− .19*
r_{yz}	+ .56***	+ .01	+ .61***	+ .33***
$r_{xy.z}$	− .23	− .17	− .19	− .17*
$r_{xz.y}$	+ .02	− .26*	− .04	− .13

* $P < .05$.
** $P < .01$.
*** $P < .005$.

any strain differences exist with respect to the magnitude of these correlations. In addition, it may be noted from these data that the correlations of the ChE variables are generally greater with the brain weight than with body weight. That the negative association does not entirely disappear between the ChE variables and body weight when brain weight is held constant (five out of the six partial correlations $r_{xz.y}$ are negative, and one is significant by itself) suggests that the negative association between the ChE variables and brain weight is probably not the only influence in the ChE-body weight correlation. Also from these data, as was found by KRECH, ROSENZWEIG and BENNETT (1958), of the two ChE variables, the subcortical ChE has the greater negative correlations with the weight variables. This greater negative association may be real, or it may be that more of the subcortical ChE variation is genetic than the cortical ChE; the lighter weight of the cortical ChE brain samples may subject those samples to more experimental weighing error.

That the total within-litter correlations support the between line associations observed is evidence for the likely possibility that selection for ChE, which was within-litter selection, has given rise to the association of these variables between the lines. It is difficult to draw any conclusions about the mode of action of selection from these data other than the tentative suggestion already made—i.e., if body weight, and hence brain weight, are manifestations of fitness, then higher ChE may produce less fit and hence less heavy animals. The dimensional changes that occur (with the possibility that brain density changes result from them alone) may be prerequisites to brain weight changes, which in turn may have to occur for some unknown reason in order to make ChE separation from selection possible. Until more exact studies are performed, which might include

histological and cytological work, the basis for these associations remains conjecture.

A positive, but insignificant, correlation between brain weight and cortical ChE was found by KRECH, ROSENZWEIG and BENNETT (1958), who also found a significant negative correlation between brain weight and subcortical ChE only. Since from their findings, and those presented here, subcortical ChE and brain weight are negatively correlated, it is not surprising that cortical ChE and brain weight are also negatively correlated as the cortical and subcortical ChE activities are themselves highly positively correlated.

Body color and color patterns: Since obvious single-gene differences were present in the stocks, a study of any possible association between them and ChE was made. The following alleles were present:

RD stock	RC stock
agouti, nonagouti	agouti, nonagouti
hooded, nonhooded	hooded, nonhooded
albino, nonalbino	albino, nonalbino
	dilution, nondilution
	shaker, nonshaker
	hairless, nonhairless

Although some tendency for fixation of alleles occurred, there was no pattern of association between direction of selection and the increase in frequency of a particular allele.

CONCLUSIONS

The correlated responses to selection, which were found in particular traits and not in others, help to elucidate the physical basis of the action of selection to produce the ChE separation. The positive association of brain density with ChE suggests that further attempts to determine percentages by weight of cell and tissue constituents which differ in density may be helpful to clarification of this physical basis. For example, the less dense, and as yet unstudied, myelin of the brain may have increased in the low lines to lower the ChE activity as it is measured. The lack of correlated response of percent protein by weight rules out the simple explanation that selection has merely altered protein percentages causing the ChE changes and brain density changes.

The negative association of brain and body weight with cortical and subcortical ChE is not easily explained. As has been already mentioned, if brain weight and body weight as well as adaptive behavior are manifestations of fitness, then the high lines may be less fit than the low lines. Corroboration for this hypothesis comes from the lesser response to selection in the high lines. Perhaps an optimum ChE level already existed in the foundation populations, and any value above that level is detrimental to the animal. However, the high lines do not appear less fit in other attributes of fitness such as sterility and number of offspring. Also, since h^2 in the low direction exceeds 1.0 in both stocks, it may be that all the ChE values of the later generations should actually be higher than observed for some reason.

The high correlated response of subcortial ChE indicates that the genes affecting the variation of ChE of the cortex are to a great extent the same genes which affect the variation of subcortical ChE. Therefore, selection has not been specific to the cortex. It would be of interest to note if ChE levels of other tissues, such as the blood, have also been altered to any extent.

It may be said that selection for cortical ChE, possibly through some cell or tissue constituent which could be characterized in one way by its density, has produced changes in ChE activity in the whole brain.

SUMMARY

Selection for high and low cortical cholinesterase activity (ChE) in the rat has been successful in two genetically heterogeneous populations of different ancestry. The ChE separation of the lines was continuous over the six generations, implying that the genetic variation of ChE was determined by several genes. The greater part of the within-litter variation of ChE was due to genetic variation (realized $h^2 = 0.78$). Some animals of each line of the S_5 generation were mated to produce two cross lines between the stocks—a high-by-high line and a low-by-low line. The ChE values of the cross lines were comparable to their respective parental lines of the S_6 generation.

Selection in the high direction was much less successful than in the low direction. Natural selection possibly may have countered artificial selection, but because of other data (h^2 in the low direction exceeded 1.0 in both stocks) it appears that other undetermined factors must have been operating.

Other traits were studied for correlated response to selection. The significant associations found were: (1) brain size negatively associated with ChE; (2) brain density positively associated with ChE; (3) brain weight correlated with body weight, both of which were negatively associated with ChE and subcortical ChE; and (4) subcortical ChE positively associated with ChE. The studies of correlated responses indicate that the successful selection for cortical ChE, possibly through some cell or tissue constituent which could be characterized in one way by its density, has produced parallel changes in ChE activity in the whole brain.

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ANALYSIS OF A HIGHLY MUTABLE GENE IN MAIZE. A MOLECULAR MODEL FOR GENE INSTABILITY

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ONE of the difficulties encountered in the study of gene mutability is the low frequency with which mutations generally occur. This frequency is significantly increased by a number of mutagenic agents such as ionizing and ultraviolet radiations and chemicals. Induced mutations result in the same types of phenotypic changes as occur by spontaneous mutation; however, it is not known whether the genic alterations involved in induced and spontaneous mutations are identical. It is well known that apparent gene mutations can result from simple deletions of small regions of the chromosome which include the locus of a particular gene (STADLER and ROMAN 1948). Most mutations are amorphs producing a phenotypic effect that can result equally as well from a change in the structure of the gene as from the complete loss of the locus. Modern biochemical techniques offer hope that such amorphs can be further analyzed to determine if enzymically inactive or modified gene products are produced.

Some genes stand out as a class in that they show a very high rate of spontaneous mutations. Such mutable genes have been described in a number of organisms but have been studied most intensively in maize. Here too, it is not known whether the mechanism involved in the mutability of unstable genes is identical with that of the low spontaneous rate mutations. With many of the unstable genes, the mutability is not autonomously controlled but requires activation by another factor, as in the case of *a-Dt* (RHOADES 1938) and *Ac-Ds* (McCLINTOCK 1951). In some instances, mutability is associated with chromosome breakage and translocation of chromosomal elements, suggesting a position effect or suppressor-like mechanism. Even spontaneous mutations are most certainly not of one class, and some could result from changes such as these.

This paper deals with the analysis of an autonomously controlled mutable gene that mutates at a very high rate to two allelic forms independent of any chromosome breakage.

Origin and phenotypic description: The mutability involves the *c* locus on the short arm of chromosome 9. This locus controls anthocyanin pigmentation in the aleurone layer of the endosperm. In the presence of the dominant alleles of genes A_1, A_2, and *R*, color is formed when *C* is also present. Homozygous recessive *c* kernels are colorless. This locus exhibits a dosage effect such that the intensity of the color produced increases with increasing doses of the dominant *C* allele.

The mutable allele is a recessive, designated c^m, which mutates to *C* and to *c*. It was found as a single, variegated kernel on an ear segregating for *c* and *wx*.

[1] Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

Linkage relationships indicate that c^m is a result of a mutation of the allele C; however, this is not necessarily the case since these two markers C and Wx are not very close, showing 26 percent recombination.

The mutations can occur at any time during the life cycle of the plant, but they can only be recognized on the ear since aleurone color alone is affected by the C gene. Late mutations occurring after fertilization appear as colored spots on the colorless kernels. The size of the spots varies from only one or a few cells to half of the kernel (Figure 1). It is not possible to recognize very late mutations to c since the phenotype of a cell carrying c^m that had not mutated is also colorless. Mutations to c occurring in the first few divisions of the endosperm appear as large, colorless areas on otherwise speckled kernels. Mutations occurring before fertilization give rise to either completely colored or colorless kernels. In the case of mutations to C, it is possible to recognize those mutations that occur during the gametophyte divisions in the female. The endosperm receives two doses of the gene from the female parent, and, quite frequently, kernels are seen that are pale self colored (one dose of C) but show patches of deeper coloration (two doses of C). Still earlier mutations give rise to sectors of either colored or colorless kernels on the ear when c^m is introduced in a cross through the female parent. The sectors, of course, also contain kernels showing the phenotype of the other homologous gene segregating at meiosis. Sectors comprising one quarter and one half of the ear are quite common as well as completely mutated ears. The latter result from mutations in the germ line occurring at any time in ontogeny between the egg and the primordial cell from which the entire ear will develop. That some

FIGURE 1.—c^m variegated seeds. Note large colorless sectors on kernels in middle row. FIGURE 2.—Ear from late mutating line. FIGURE 3.—Ear from intermediate mutating line.

of these completely mutated ears result from postzygotic mutations has been established by scoring two ears from the same plant, one of which is completely mutated, whereas the other shows sectors and variegated kernels.

Characterization of mutation: Linkage relationships have placed the mutable gene at the *c* locus (Table 1). It is difficult to determine exact recombination values in ears showing germinal mutations since it is not possible to recognize all the exchanges. From a backcross of c^m *Wx/c wx* × *c wx/c wx*, *c Wx* kernels can result either from c^m *Wx* → *c Wx* mutations or from recombination. However, this is not a factor when ears showing only late mutations are used for linkage studies. In these ears, which show no germinal mutations, every c^m kernel is speckled. The $c^m/c^m/c$ kernels show colored spots on a colorless background, whereas $c^m/c^m/C$ kernels show dark spots on a pale colored background.

As far as can be determined by genetic studies, the mutability is autonomously controlled. This is also best shown with the late mutating lines where every kernel that has received the c^m allele can be recognized by its speckled appearance. Selfing of c^m/c plants produced 75 percent speckled kernels. Successive backcrossing of c^m/c to a number of c/c testers gave 50 percent speckled kernels in each cross. Thus if the mutability of c^m is dependent on activation by another locus, the activator was not segregating or was present in the homozygous condition in all the plants used in these crosses.

The mutations of the c^m allele fit all the criteria of true genic mutations. It mutates to either one of two allelic forms, *C* and *c*. Once such a mutation has occurred, the new allele is completely stable; no back mutations either to the c^m condition or to the other allelic form have been observed to date. Mutations to *c* can be recognized and distinguished from c^m in that they show no changes to *C*. Kernels heterozygous for c^m and *C* are completely colored, indicating that c^m does not mutate to *I*, a third allele at this locus that is a dominant inhibitor of color. Any such mutation would give rise to a colorless area on the kernel.

The mutations to *C* and *c* are not associated with detectable chromosome breakage or aberrations as is the case with some *Ac-Ds* material (McCLINTOCK 1951). No chromosome aberrations involving the region of the *c* locus have been found in pachytene of plants carrying the c^m gene. Likewise, none have been found in plants carrying *C* or *c* resulting from germinal mutation of c^m. A sharper criterion is the absence of the breakage-fusion-bridge cycle in endosperm showing postfertilization mutations. This is deduced from the pattern of

TABLE 1

Distribution of phenotypic classes among progeny of c^mWx/cwx × cwx/cwx *crosses*

State of c^m	c^mWx	c^mwx	*CWx*	*Cwx*	*cWx*	*cwx*	Percent crossing over	Percent *c*
Late mutating	2512	779	0	0	773	2542	23.5	50.2
Intermediate mutating	1805	488	79	14	856	2078	...	55.2
Early mutating	167	50	158	42	776	1056	...	81.2

variegation. All of the colored spots on the kernels resulting from mutations of c^m to C are solid colored and not broken up into colored and colorless areas. Furthermore, kernels of the constitution $c^mWx/cwx/cwx$ are completely starchy and show no waxy spots. Association of the mutation with chromosome breakage in the endosperm would result in bridge formation through fusion of broken sister chromatids after duplication and variegation for markers proximal to the point of breakage (McCLINTOCK 1941).

The only aberration involving chromosome 9 found in this material was a reverse duplication of the short arm, but here the point of initial breakage was far removed from *c*, just proximal to the terminal knob. It should also be mentioned, for comparison with the *Ac-Ds* system of McCLINTOCK, that during the five years that this problem has been under investigation, no other unstable genes involving other loci have been detected in this line.

States of c^m: As was mentioned earlier, mutation of c^m can occur at any time during the life cycle in the sporophyte as well as the endosperm. However, c^m plants can be divided into three fairly distinct classes differing in the timing of the mutational events. The three classes, designated states of c^m after McCLINTOCK's terminology, are the late, intermediate, and early mutating states. For the investigation of the c^m states, it was desirable to use c^m as the female parent in the crosses so that early mutations could be recognized as sectors on the ears. Also the crosses $c^m/c \times c/c$ were made with c^m heterozygous with a stable *c* so that the state of a single c^m gene could be followed. A change in state of one of the genes in a c^m homozygote would make the analysis difficult. Thus, independently of any mutational events, half the kernels on the ears are colorless as the result of segregation of the stable *c* allele. In the late mutating state, the mutations occur almost exclusively only after fertilization (Figure 2). Occasionally a single self-colored kernel is found on an ear. The kernels receiving the c^m gene are variegated, showing various sized patches of color. In such crosses, half the kernels are colorless and half variegated, indicating no germinal mutations to either *C* or *c*. A few ears were found with an excess of colorless kernels. There were no self-colored seeds on these ears, but many of the variegated seeds showed only a few tiny specks of color. These ears were also segregating for *Wx* and *wx* with c^m linked to *Wx*, and the colorless starchy class was in excess. These kernels could have resulted from germinal mutations to *c*. However, on further progeny testing, most of these seeds were shown to still be carrying the c^m allele. As is the case when kernels carry three doses of *Ac*, the timing of the mutational events was so late that the kernels had completed development before the mutations could occur. The corrected counts showed 50 percent c^m and 50 percent *c* kernels.

The majority of the colored kernels on ears in the intermediate mutation state are variegated, but an average of about five percent are self-colored (Figure 3). Large colored patches are common on the variegated kernels as contrasted to the late state where most of the colored patches are small. The proportion of colorless kernels is somewhat higher than 50 percent indicating germinal mutations to *c* (Table 1).

Ears in the early mutating state show a large excess of colorless kernels, and many of the colored kernels are self-colored (Table 1). However, some variegated kernels showing very tiny specks of color are still found. Large sectors on the ears containing only colorless kernels, or a mixture of self-colored and colorless but lacking variegated seeds, are frequently seen (Figure 4).

The germinally mutated self-colored and colorless seeds from these ears were tested for stability by backcrossing to *c* testers. Crosses involving plants grown from the colored seeds gave almost exclusively ears segregating only self-colored and colorless seeds in equal proportions. Crosses of plants grown from the colorless seeds gave almost solely completely colorless ears. A few ears showing c^m variegation were found in each cross. This was because the original classification of the seed was based on the phenotype of the endosperm, and mutations in the gametophyte divisions could cause the endosperm and the embryo to be genotypically different. Similarly, some kernels with variegated endosperms showed germinal mutations in the embryo, giving rise to either completely colorless ears or ears with 50 percent colored and 50 percent colorless kernels.

Mutations of c^m to *c* are about four times as frequent as the mutations to *C*. This is calculated from the proportion of the self-colored to the excess of colorless seeds over the 50 percent resulting from the segregation of the *c* allele in the early mutating line crosses.

The state of c^m determining the timing of the mutations is controlled by the c^m locus itself. This is best shown with the late state, which is highly stable. The intermediate and early states are unstable and revert to one or the other form. Plants carrying the late mutating state of c^m were backcrossed to a number of different *c*/*c* tester lines as well as to *I*/*c* plants, and no segregations for state of mutability were observed in the progeny. In another test, state heterozygotes of the following type were synthesized, $c^{m(late)}Wx/c^{m(intermediate)}wx$, and backcrossed to cwx/cwx^{δ}. Of the 15 self-colored seeds on the ear, 11 were *wx* and

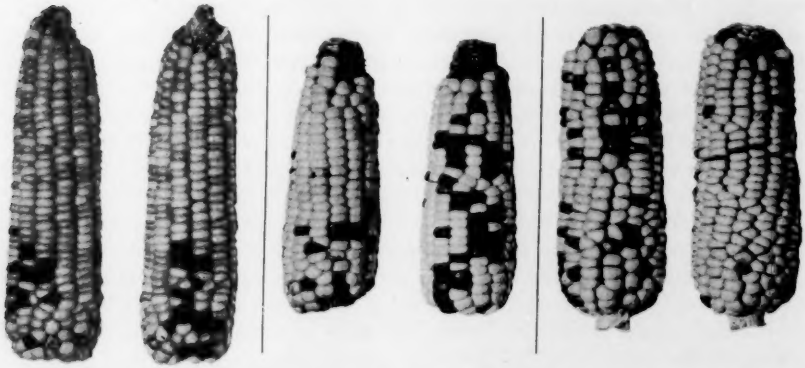

FIGURE 4.—Ears from early mutating lines. Both sides of each ear are shown to indicate sectoring.

only four were *Wx;* the latter probably were the result of recombination between the $c^{m(late)}$ allele or its mutant *C* derivative and *Wx*. Of the 188 variegated kernels, the *wx* seeds showed predominantly larger spots than did their *Wx* sibs, reflecting the intermediate state of c^m. Thus, both c^m and the factor determining the state of c^m are linked to waxy. The state of a c^m gene is best determined not by the phenotype of the variegated seed carrying this gene, since some kernels showing only very late mutations are found even in ears showing very early mutations, but by proportion of germinally mutated kernels on an ear. Therefore, the *wx* and *Wx* variegated seeds from the above cross were planted and backcrossed to *cwx/cwx*. The results corroborated the evidence obtained from kernel scoring. Seventy-three ears were obtained from the *Wx* seeds, of which 67 had none or less than one percent self-colored seeds. Of the 45 *wx* ears, only 18 had none or less than one percent self-colored kernels.

The strongest evidence indicating that the state of c^m is controlled by the c^m locus itself comes from the following experiment. Plants were made heterozygous for a $c^{m(late)}$ and a stable *c* derived by mutation from a c^m in the early mutating state. These plants were backcrossed to *c* tester plants, and all the ears were found to be in the late state. Half of the kernels were colorless and the other half variegated for small spots of color.

The plant obtained from the original variegated kernel, heterozygous for c^m and *c*, was self-pollinated and used as the male parent in a number of backcrosses to *c*. The selfed progeny segregated 43 colored, 57 variegated, and 276 colorless kernels. The backcross progeny consisted of 135 colored, 293 variegated, and 1235 colorless kernels. Thus, the original seed was in the early mutating state and in the next generation gave rise to the late and intermediate mutating states. The late mutating state is quite stable, as has been mentioned previously, in that seeds from ears in this state produce progeny ears that are predominantly in the late state. In one experiment, of 290 ears resulting from planting and backcrossing seeds in the late state, 242 showed no self-colored germinals; 40 had less than three percent self-colored in the colored class, the remainder being variegated; eight ears showed between three and ten percent self-colored; and none had over ten percent. The intermediate mutating line shows frequent change of state. Of 380 ears from seeds in the intermediate mutating state, 56 showed no self-colored seeds, 124 had less than three percent, 158 had between three and ten percent, and 27 ears had over ten percent self-colored seeds in the colored class. Fifteen ears were either completely colorless or segregating for only self-colored and colorless seeds, indicating either mutations in the egg or in any cell during ontogeny from which the entire ear had arisen. Of 107 ears from variegated seeds of the early mutating state, ten had no self-colored seeds, four had less than three percent, six had between three and ten percent, 58 had over ten percent, and 29 ears were completely mutated. The ears that showed over ten percent self-colored had a high excess of colorless seeds, and most of the colored kernels were in sectors on the ears.

Analysis of c^m *mutability and behavior:* Before analyzing the mutational behavior of c^m it is necessary to briefly review the life cycle of the maize plant. The

2n zygote develops into the sporophyte. At meiosis, four haploid spores are produced from each spore mother cell. In the male all of these spores are functional. The haploid nucleus undergoes one division to form the generative and tube nuclei. The generative nucleus then divides again giving rise to the two sperm, so that the mature pollen grain contains three nuclei—the tube nucleus, which governs the growth of the pollen tube, and two sperm. On the female side only one of the spores functions; the other three degenerate. The haploid nucleus undergoes three divisions so that the embryo sac contains eight haploid nuclei, four at each end. Two nuclei migrate to the central portion of the embryo sac to form the polar bodies. One of the nuclei remaining at the micropylar end of the embryo sac becomes the egg. Double fertilization occurs in maize. One of the sperm fertilizes the egg to form the 2n zygote that develops into the sporophyte completing the life cycle. The other sperm fertilizes the polar bodies to form the 3n endosperm. It can thus be considered that a dichotomy occurs in the development of the kernel, forming the embryo and the endosperm, with only one of these branches, the embryo, involved in the continuity of the life cycle.

Analysis of the mutational behavior of the c^m gene has established the following chain of events. After fertilization of the egg, the c^m gene is stable and does not mutate. At some period during the life cycle, a change occurs to the unstable condition so that the gene becomes highly mutable. This does not necessarily mean that the gene mutates at this time, only that it is in a condition where mutations can occur with a high frequency. The gene remains in this mutable state until fertilization. If, by fertilization, the c^m gene has not mutated to either the *C* or *c* form, it reverts to the nonmutable condition in the 2n zygote thus completing the cycle. However, after fertilization in the endosperm, the gene remains in its highly mutable condition. Thus, on ears showing large sectors of colorless or colored kernels, one still finds some variegated seeds with very late mutations in the endosperm. The state of the c^m gene determines the time during the life cycle at which the switch from the stable to the unstable condition occurs. In the early state it occurs during the early development of the sporophyte. In the intermediate state it occurs at about meiosis. In the late state the switch occurs late in development, after fertilization, so that the endosperm is variegated but germinal mutation is extremely rare.

This alternation between the mutable and stable condition is deduced from the following observations. In plants of the intermediate state, the c^m gene begins to mutate at some time before fertilization. If the variegated kernels from such plants showing only postfertilization mutations, are grown and backcrossed to recessive *c* plants, the resulting ears are again typical of the intermediate class, i.e., showing some scattered self-colored kernels on the ears although the majority of the colored kernels are variegated. If the c^m gene had continued to mutate after fertilization in the embryo as well as in the endosperm, very early mutations would have occurred giving rise almost exclusively to either completely mutated ears or to ears that showed very large sectors of colored or colorless kernels. The fact that the great majority of the ears were still of the intermediate class indicates that there had been a reversion to the stable condition in the embryo and

not a return to the mutable condition until just before fertilization in the next generation.

DISCUSSION

In replication, the c^m allele can either persist in the unstable form or mutate to the stable *C* or stable *c* condition. The time during ontogeny at which c^m begins to mutate also seems to be autonomously controlled by the particular c^m allele that is mutating. Thus, there are at least five and possibly nine allelic conditions at this locus. The gene can be in the late, intermediate, or early mutating form, and any one of these can remain as c^m or become *C* or *c*. The further alternation between the mutable and the nonmutable state during the life cycle adds another complication. However, the latter probably does not reflect a change in the gene but simply a change in the physiological condition of the plant tissue during growth. The results suggest that the gene mutates in response to the cellular environment. We postulate that the gene is stabilized in the embryonic environment but can mutate in the older tissue. At fertilization, the embryonic environment is once again restored and the gene is again stabilized. In this respect the endosperm behaves as old tissue since in this tissue the c^m gene continues to mutate. Thus it is not simply the act of fertilization that is responsible for the reversion to the embryonic state since fertilization is essential for both embryo and endosperm development. A similar situation is found in the fusion of broken ends of sister chromatids (McCLINTOCK 1941). The chromatid bridge-breakage-fusion cycle persists in the gametophyte and in the endosperm after fertilization but stops in the embryo where broken sister chromatids do not fuse.

The c^m mutations can be of two types: either involve alteration in the *C* gene itself or involve a closely linked suppressor, as is the case with McCLINTOCK's *Ac-Ds* phenomenon. On the latter notion c^m would be represented as a complex composed of *C* and a suppressor *S*. A mutation to *C* would result from deletion of the suppressor alone, but a change to the stable *c* condition would have to be the result of a deletion of both the suppressor and the *C* gene or a stabilization of the suppressor such that it is never transposed (Figure 5). Although precedence for such a mechanism exists in the case of McCLINTOCK's system, direct evidence is lacking here in that there is absolutely no indication of any associated chromosome breakage. One would have to postulate that when *S* or the *SC* com-

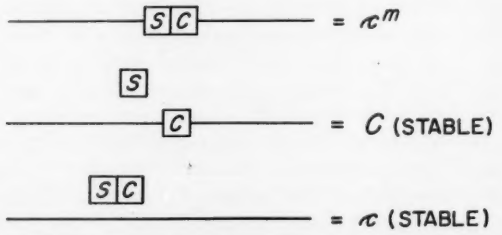

FIGURE 5.—Diagrammatic representation of the suppressor hypothesis of mutability.

plex is deleted, restitution to reform the original chromosome configuration occurs in 100 percent of the cases.

The other alternative is that the mutations to the *C* and *c* stable alleles result from changes in the *C* gene itself. At this time one can only speculate as to the mechanism involved. If we accept the assumption that genetic information resides in the sequence and arrangement of nucleotides in DNA, the three alleles c^m, *C*, and *c* would represent three different nucleotide sequences. Only one of these sequences can specify the correct enzyme necessary for anthocyanin formation in the aleurone since in the absence of mutation the c^m gene gives rise to colorless tissue. The c^m DNA would have to be capable of undergoing a large number of exact duplications but yet begin to misreplicate or mutate at some later time, producing two new DNA sequences, *C* and *c*, with a frequency as high as eight percent per cell division.

We propose that the c^m condition represents an illegitimate base pairing in the WATSON-CRICK (1953a) DNA model such that an alteration in one of the bases could result in legitimate complementary pairing accompanied by stabilization in the form of either *C* or *c*. Such a scheme is not possible according to the replication mechanism proposed by WATSON and CRICK (1953b) where the daughter DNA molecules are composed of an old and a new strand. The first duplication of the DNA would already form the complementary base pairs and the mutable condition could not be perpetuated. However, such a scheme is consistent with the modification of the WATSON-CRICK model proposed earlier (SCHWARTZ 1955, 1958). According to this modification, the two strands of the DNA molecule are connected at one end so that effectively the double helix is composed of only a single strand coiled back on itself. On replication a double length DNA molecule is produced composed of one old and one new strand. Each strand then coils back on itself to give the intact old DNA molecule and a completely new molecule. Thus, according to this model, an illegitimate base pair would, as a result of the replication process, maintain its noncomplementary configuration, and furthermore replicate another illegitimate pair at the identical position in the newly synthesized DNA molecule. As long as such exact replications occur, the gene would remain in the stable c^m form. Reversion to the mutable state could take place when the cellular environment was such that misreplication or base or nucleotide exchange occurred in the DNA in the absence of replication (ZAMENHOF, REINER, DEGIOVANNI, and RICH 1956; ZAMENHOF, RICH, and DEGIOVANNI, 1959). Since the illegitimate pair of bases would not be hydrogen bonded to each other, any base switch that would result in the formation of a complementary base pairing would be stabilized by the formation of hydrogen bonds between the bases. Stabilization could occur to give either the original *C* or altered *c* sequences (Figure 6). The differential mutation rate of c^m to *C* and *c* may reflect differences in the probability of base exchange. Since the bonding force between guanine-cytosine is greater than that between adenine-thymine, the conversion of an illegitimate base pair to a guanine-cytosine pair might be favored.

The early, intermediate, and late states of the c^m gene reflect the time at which

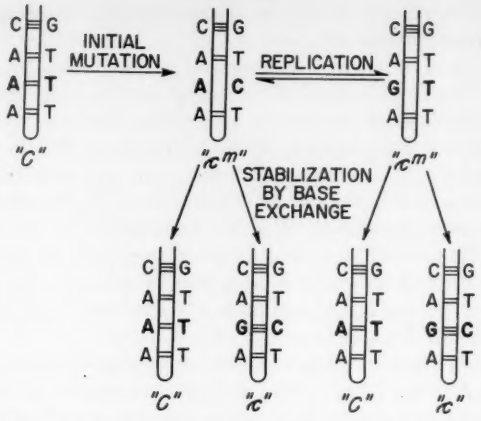

FIGURE 6.—Diagrammatic representation of the illegitimate base pair hypothesis.

such base exchanges could occur and have a bearing on the question as to why such unstable forms are not more frequent. Only those mutations that for some reason or another were associated with a block in base exchanges could become unstable. Only by constant selection of these gametes that had not mutated have we been able to keep the unstable c^m line going. In the absence of such selection the gene would very quickly become stabilized in a population as *C* or *c*. If the base exchange occurred soon after the original mutational event, the gene would become stabilized before it could be recognized as an unstable mutant. Some apparently stable mutants, however, may be of the illegitimate base configuration type where the reversions to the mutable condition occur so late in ontogeny as to be unrecognizable, similar to the effect of three doses of *Ac* in the McCLINTOCK system. This hypothesis considers only mutations of c^m to *C* and *c* and not the changes in state. The latter may result from independent epigenetic changes in a closely linked region.

This modification of the WATSON-CRICK model makes DNA replication consistent with two lines of evidence that, at first glance, appear to be contradictory: first, *in vitro* DNA synthesis involves a single strand serving as template for the replication of the complementary strand (BOLLUM 1959, 1960a,b); and second, as a result of DNA replication, a completely new DNA molecule is formed with the old molecule remaining intact. The latter is deduced from an evaluation of the results presented by CAVALIERI, ROSENBERG, and DEUTSCH (1959) and MESELSON and STAHL (1958). By the cesium chloride gradient centrifugation technique, MESELSON and STAHL showed that N^{15}-labeled bacteria form hybrid $N^{14}N^{15}$ DNA when allowed to replicate once in N^{14} medium. When heated in the cesium chloride, the DNA separated into two units, one of which was all N^{15} and the other all N^{14}. A semiconservative replication mechanism, in which the

daughter molecules are half old and half new, requires that the separated units represent single strands. However, CAVALIERI's results suggest that these units are still double stranded, a finding consistent with the conservative replication scheme where a double-stranded DNA molecule remains intact on replication and forms a completely new double-stranded molecule.

Actually, the modified DNA replication scheme (SCHWARTZ 1955) can be either conservative or semiconservative, depending on the starting point in the replication cycle (Figure 7), i.e., the DNA configuration in the resting nucleus at the initiation of the replication process. If the starting point is at (a) then replication is conservative, whereas if it is at (c) it is semiconservative.

To date all attempts to stabilize the c^m gene experimentally have failed. These included irradiating the c^m pollen with ultraviolet and ionizing radiations and treating c^m seeds with ionizing radiations and high-temperature shock. The stability of the late mutating line as compared with the intermediate and early lines suggests that changes in state are associated with or occur at the same time as mutations of c^m to the *C* or *c* allelic forms.

SUMMARY

A genetic analysis of a highly mutable gene in maize is presented. The unstable gene is a recessive *c*, designated c^m, that mutates at a very high rate to both *C* and *c*. Once mutated, the gene is completely stable. The c^m allele can mutate at any time during ontogeny. Three states of the gene are found relating to the time during ontogeny at which the gene begins to mutate. The c^m allele shows cyclic changes in mutability alternating between the stable and unstable condition. A molecular model for the instability is presented based on illegitimate base pair configurations in DNA.

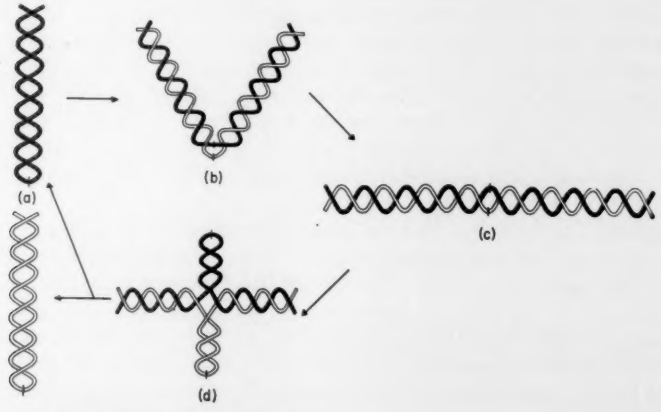

FIGURE 7.—Modified DNA replication scheme.

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A COMPARISON OF THE EFFECT OF LETHAL AND DETRIMENTAL CHROMOSOMES FROM DROSOPHILA POPULATIONS[1]

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THIS study was undertaken in an attempt to determine whether the effects of recurrent mutation on the population and the deleterious effects of inbreeding are due primarily to a small number of genes of major effect or to the cumulative activity of a number of genes with individually small effects. It has been known since the work of TIMOFEEFF-RESSOVSKY (1935) and KERKIS (1938) that X-ray-induced mutations, and presumably spontaneous mutations as well, consist not only of lethals and near-lethals but include many with minor effects. It is likely that such a study would underestimate the number of minor genes for two reasons: (1) X-ray-induced changes include more chromosome breakages which are likely to be more drastic in their effect, and (2) mutants whose effects are very small would not be detected in such an experiment for lack of statistical resolving power.

This experiment consisted of the extraction of 465 second chromosomes from wild and from long-continued laboratory populations and the measurement of their effect on viability when homozygous and in random heterozygous combinations. In this way we obtain the distribution of homozygous deleterious effects among these chromosomes. This can be compared with similar data on newly induced mutations, to yield information on the relative persistance in the population of mutants of different homozygous viability classes.

Genes with a very slight detrimental effect cannot be detected individually, but their over-all influence can be assessed by applying the concept of the genetic load (MORTON, CROW, and MULLER 1956). The genetic load due to, say, mutation is the extent to which the population fitness (or whatever is being measured) is reduced in comparison to what it would be if mutation were not occurring. In addition to the mutation load there are the segregation load, due to segregation of homozygotes at loci where a heterozygote is favored, and other loads (CROW 1958).

In order to maximize the effect of mutations with small effects, the measurements were made on natural populations or on long continued cage populations which had been maintained long enough to be somewhere near equilibrium between selection and mutation. Similar measurements have been made by others (TH. DOBZHANSKY, HOLZ, and SPASSKY 1942; TH. DOBZHANSKY and SPASSKY 1953, 1954; DUBININ 1946; PAVAN, CORDEIRO, N. DOBZHANSKY, TH. DOBZHANSKY, MALOGOLOWKIN, SPASSKY, and WEDEL 1951; GOLDSCHMIDT

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1951; IVES 1945), though the method of analysis differed from that which we employ here. Studies on the distribution of lethals, sublethals, and detrimentals, among newly arising mutants have been made by TIMOFEEFF-RESSOVSKY (1935), KERKIS (1938), KÄFER (1952), FALK (1955), and BONNIER and JONSSON (1957).

EXPERIMENTAL METHODS

The chromosomes analyzed came from two sources: (1) a wild population collected in Madison, Wisconsin (Population I); and (2) a cage that had been maintained as a large population for several years by DR. BRUCE WALLACE as a control for his radiation experiments (IIa and IIb).

Males from these populations were crossed individually with a *Cy/cn bw* female (*Cy* = Curly wing, *cn* = cinnabar eyes, *bw* = brown eyes). A single Curly male (*Cy*/+) from each culture was crossed with *SM1*/bw^D female. (*SM1* is a chromosome with a pericentric inversion in addition to the usual Curly inversions, which prevent virtually all crossing over on the second chromosome. This chromosome produces the Curly wing phenotype in heterozygotes and is lethal when homozygous; bw^D is a dominant brown eye color.)

To produce flies homozygous for a second chromosome extracted from the population, *SM1* (*Cy*)/+ females from the above crosses were mated with their bw^D/+ brothers in individual pairs. There are four classes of progeny, expected in equal numbers, *Cy*/bw^D, *Cy*/+, bw^D/+, and +/+. The last class has two identical chromosomes derived from the same chromosome in the original male.

Flies carrying random combinations of wild chromosomes were obtained by following the same crossing procedure, except that the last mating was between flies derived from different wild males. The extracted chromosomes were cyclically permuted in the crosses, i.e., 1×2, 2×3, 3×4, . . . $(n-1) \times n$, $n \times 1$.

This system of crossing was designed so that each chromosome would be represented the same number of times in the heterozygous (random) combinations as in the homozygous (identical) combinations. This ideal was not quite fully achieved, for despite carrying duplicate cultures to meet such contingencies, some crosses were not completed due to sterility or inviability of the cultures. Heterozygous and homozygous combinations were made at the same time and run as nearly as possible under identical conditions, in order to minimize any confounding effect of secular fluctuations in the laboratory environment.

All cultures were kept in a constant temperature room at 24–26°C. The medium used was a standard corn meal, molasses, yeast, agar type sprayed with live yeast and with 0.5 percent propionic acid as a mold inhibitor.

Each pair of parents was transferred to a fresh vial, usually after four days. Counts were made on the 12th, 15th, and 18th day after mating. The values in Table 2 are the averages of the total number in both vials.

DATA

In Table 1 and Figure 1 are given the distributions of the viabilities of +/+ flies, the viabilities being expressed as the ratio of the +/+ class to the average

of the $Cy/+$ and $bw^D/+$ classes. The Cy/bw^D class was omitted from the calculations because of its reduced frequency in all experiments.

As in all other species of Drosophila examined, the distributions for the homozygotes are bimodal. One peak lies just below the normal viability, showing that most of the homozygous individuals are not as fit as heterozygotes. This must mean that in an equilibrium population there are many deleterious genes with very slight effects, bordering closely on the normal. The second peak of the distribution lies at the level of complete lethality. In addition to these chromosomes which kill all of the homozygotes, there are a few which allow a very small number of $+/+$ individuals to survive, usually less than ten percent of the normal number. Moreover, in many cultures of this type the $+/+$ flies emerged late. For these reasons it is convenient to include with the lethals those chromosomes which gave less than ten percent as many wild type as the control mean. The frequencies of lethals in the three tests were 26.0, 24.0, and 23.9 percent, in general agreement with values found by other investigators for second chromosomes of *Drosophila melanogaster*.

The mean numbers of flies per pair of parents are given in Table 2. An absence of any appreciable effect of crowding is indicated by the lack of any negative correlation between the numbers in the Cy/bw^D, $Cy/+$, and $bw^D/+$ classes and the $+/+$ class, despite absolute changes in the $+/+$ frequencies in homozygotes and heterozygotes. Differences in numbers of flies per culture among the three experiments may be accounted for by the tests having been performed under different food and laboratory conditions, in addition to having involved flies from different strains.

In Table 2 are also presented the mean viability ratios for heterozygotes (A), homozygotes (B), and nonlethal homozygotes (C). The mean ratios for all three experiments were obtained by weighting each value by the reciprocal of its variance.[2]

By observing the mean viability ratios, we see that a population suddenly made homozygous for all second chromosomes would retain, under our experimental conditions, only about 63 percent of the normal viability. Made homozygous for all second chromosomes excepting those carrying lethals, the population would retain about 84 percent normal viability. The reduction in viability in the latter case is due to a group of genes with relatively mild effect. Since the boundaries of these are not discrete and are definable only by a convention, it is best to group them all together as "detrimentals," which here include the "semilethals" mentioned by other authors. Estimation of the frequency of detrimentals poses a difficult problem since this group of mutants merges imperceptibly with the normals. Therefore, rather than establishing arbitrary limits to the detri-

[2] HALDANE (1956) has shown that estimates of viability such as A and B are biased. If n and N are the numbers of two classes, the viability estimate, $n/(N+1)$ is almost unbiased, and for this reason is preferable to the uncorrected estimate, n/N. However, we are interested primarily in the ratio (in fact, the log of the ratio) of two such viability estimates. The log of A/B is the same whether A and B are calculated as the average of $(n/N+1)/(m/M+1)$ or of $(n/N)/(m/M)$, provided that the numbers N and M are approximately the same magnitude. Since that is true in this study, the correction would have a negligible effect.

TABLE 1

Distribution of viability of homozygotes and heterozygotes for second chromosomes, expressed in viability ratios, i.e., $+/+ : \frac{1}{2}(\mathrm{Cy}/+ + \mathrm{bw^D}/+)$. *The figures indicate numbers of chromosomes that fall in the different viability classes*

	Viability ratios																		
Experiment	0	.10	.20	.30	.40	.50	.60	.70	.80	.90	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	Total
I-homozygotes	58	2	3	3	5	8	8	16	32	34	25	16	9	5	5	1	1	..	231
IIa. homozygotes	23	6	1	2	4	3	2	10	13	17	16	13	8	2	1	..	..	..	121
IIb. homozygotes	25	2	3	0	0	2	2	5	13	21	23	13	1	3	..	..	..	..	113
Total	106	10	7	5	9	13	12	31	58	72	64	42	18	10	6	1	1	..	465
I-heterozygotes*	..	..	..	..	1	2	2	8	23	22	41	36	22	28	13	12	2	2	216
IIa. heterozygotes	..	..	..	..	..	..	..	3	9	20	26	27	17	11	2	2	0	1	118
IIb. heterozygotes	..	..	..	..	..	..	..	2	9	19	29	33	15	5	1	..	..	..	113
Total*	..	..	..	..	1	2	2	13	41	61	96	96	54	44	16	14	2	3	447

* Two outlying observations 2.18 and 2.50 omitted from this line.

TABLE 2

Mean genotype numbers and viability ratios in three experiments

	n	Cy/bw^D	$Cy/+$	$bw^D/+$	$+/+$	Viability ratio* $+/+ : \frac{1}{2}(Cy/+ + bw^D/+)$
			Experiment I (Madison)			
Heterozygotes	216	40.10	45.76	47.59	47.84	1.049 ± .018
Homozygotes	231	40.49	48.00	47.45	28.29	.614 ± .028
Nonlethal homozygotes	171	38.97	47.04	46.60	38.19	.829 ± .020
			Experiment IIa (Wallace)			
Heterozygotes	118	51.08	60.68	57.80	59.63	1.012 ± .016
Homozygotes	121	53.36	61.68	60.31	37.96	.641 ± .038
Nonlethal homozygotes	92	53.14	60.20	60.46	49.79	.841 ± .025
			Experiment IIb (Wallace)			
Heterozygotes	113	70.62	81.00	73.58	75.91	.985 ± .013
Homozygotes	113	68.36	78.81	74.01	48.74	.656 ± .038
Nonlethal homozygotes	86	65.71	76.90	72.17	63.88	.860 ± .022

* Viability ratio for all experiments.
Heterozygotes (A) 1.008±.0088
Homozygotes (B) .632±.0194
Nonlethal homozygotes (C) .842±.0127.

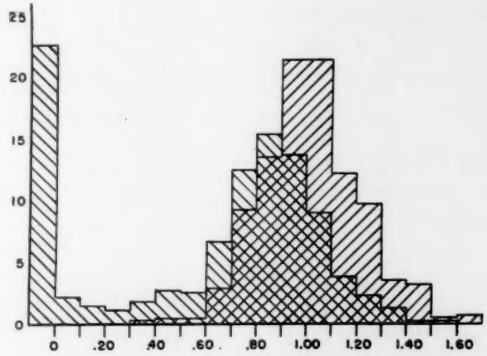

FIGURE 1.—Distribution of frequencies of viability ratios, $+/+ : \frac{1}{2}(+/Cy + bw^D/+)$. Abscissa: viability ratios. Ordinate: frequencies of chromosomes, in percent. ///// = heterozygotes. Reverse hatch = homozygotes. Crosshatched area is area of overlap.

mental class, it is desirable to have a method which takes into account even those detrimentals with extremely minute effect. This may be achieved by measuring the genetic load, which gives an estimate of the total effect of all grades of mutant genes upon a population.

METHODS OF ANALYSIS

The homozygous load may be estimated from the data in the following manner. Let us assume (1) a series of independently acting lethals with frequencies Q_i and probabilities of death S_i; (2) a series of independently acting detrimentals with frequencies q_i and probabilities of death s_i; (3) a series of independent heterozygous or environmental factors with frequencies X_i and probabilities of death H_i. This includes effects due to chance homozygosis of alleles in independent chromosomes.

On this model the probabilities of surviving the adverse effects of these factors are as follows:

The fraction of survivors among:

(1) heterozygotes $= A = (1 - X_1H_1)(1 - X_2H_2) \ldots\ldots$
$= \Pi\,(1 - X_iH_i)$.

If the number of factors is large and the separate probabilities small, this is approximately

$$e^{-\Sigma X_iH_i}$$

(2) homozygotes $= B = (1 - X_1H_1)(1 - X_2H_2) \ldots (1 - Q_1S_1)(1 - Q_2S_2) \ldots (1 - q_1s_1)(1 - q_2s_2) \ldots$

$$= \Pi\,(1 - X_iH_i)(1 - Q_iS_i)(1 - q_is_i).$$

$$\sim e^{-\Sigma X_iH_i - \Sigma Q_iS_i - \Sigma q_is_i}$$

(3) nonlethal homozygotes $= C = (1 - X_1H_1)\ (1 - X_2H_2) \ldots (1 - q_1s_1)(1 - q_2s_2)$

$$= \Pi\,(1 - X_iH_i)(1 - q_is_i)$$
$$- \Sigma X_iH_i - \Sigma q_is_i$$
$$\sim e$$

Let T = total load $= \Sigma q_is_i + \Sigma Q_iS_i$
D = detrimental load $= \Sigma q_is_i$
L = lethal load $= \Sigma Q_iS_i$

Then,

$$\frac{B}{A} = \frac{e^{-\Sigma X_iH_i - \Sigma q_is_i - \Sigma Q_iS_i}}{e^{-\Sigma X_iH_i}} = e^{-\Sigma q_is_i - \Sigma Q_iS_i} = e^{-T}$$

$$\frac{B}{C} = \frac{e^{-\Sigma X_iH_i - \Sigma q_is_i - \Sigma Q_iS_i}}{e^{-\Sigma X_iH_i - \Sigma q_is_i}} = e^{-\Sigma Q_iS_i} = e^{-L}$$

$$\frac{C}{A} = \frac{e^{-\Sigma X_iH_i - \Sigma q_is_i}}{e^{-\Sigma X_iH_i}} = e^{-\Sigma q_is_i} = e^{-D}$$

Thus, $T = ln\ A - ln\ B$
$L = ln\ C - ln\ B$
$D = ln\ A - ln\ C$

From Table 2 we see that the average value of T is .467. Thus we can say that the typical second chromosome in this population carries .467 lethal equivalents of which .287 are lethals and .180 are nonlethal detrimentals.

We are especially interested in computing the theoretical ratio of D to L for various specific models of gene action, and comparing these with observed results. In particular, the comparison of the D:L ratio in newly arisen mutations with that of chromosomes from natural populations is of interest.

The expectations may be derived as follows.

New mutations

In a group of new mutations, not yet exposed to selective elimination, the ratio of detrimentals to lethals will be determined solely by the total rates at which such mutants occur. To determine the load we weight each mutant by the amount of harmful effect it causes. Thus the D:L ratio is $\Sigma us/\Sigma US$, where u is the total rate of mutation to detrimental alleles at a locus and s is the average homozygous effect of detrimentals at this locus. U and S refer to the same quantities for lethals. The summations are over all relevant loci. If the same locus produces both detrimental and lethal alleles, the contribution may be thought of as divided proportionately between the two loads. If the mutation rate and the selective disadvantage are independent, the D:L ratio becomes $\bar{n}\bar{u}\bar{s}/N\bar{U}\bar{S}$ or, since this

TABLE 3

The expected Detrimental:Lethal load ratio for various levels of dominance

	D:L Ratio		D:L Ratio (equilibrium)
	General	u and s independent	D:L Ratio (new mutants)
New mutants	$\Sigma us/\Sigma US$	$\bar{n}\bar{s}/N\bar{S}$	
Equilibrium	$\Sigma(u/h)/\Sigma(U/H)$		
I. $h > 0$			
a. h constant	$\Sigma u/\Sigma U$	n/N	$\bar{S}/\bar{s}$
b. hs constant	$\Sigma us/\Sigma US$	$\bar{n}\bar{s}/N\bar{S}$	1
c. h/s constant	$\Sigma(u/s)/\Sigma(U/S)$	$n\bar{S}/N\bar{s}$	$(\bar{S}/\bar{s})^2$
II. $h = 0$	$\Sigma\sqrt{us}/\Sigma\sqrt{US}$	$n\sqrt{\bar{s}}/N\sqrt{\bar{S}}$	$(\bar{S}/\bar{s})^{1/2}$
III. $h < 0$			
a. k alleles	$\Sigma\tilde{s}/\Sigma\tilde{S}$	$n\tilde{s}/N\tilde{S}$	1
b. 2 alleles $s_1 >> s_2 = \text{const.}$		n/N	$\bar{S}/\bar{s}$

u,U—mutation rate (total rate per locus to all mutant alleles).
s, S—proportional reduction in fitness of the homozygote.
h, H—a measure of dominance, ranges from one for completely dominant mutant, through zero for completely recessive, to negative values for overdominant locus.
n, N—number of relevant loci.
$\tilde{}$ indicates the harmonic mean of s.
Lower case letters refer to detrimentals, upper case to lethals.

independence implies that $\bar{u} = \bar{U}$, $n\bar{s}/N\bar{S}$. N and n are the number of lethal- and detrimental-producing loci, and $\bar{S}$ and $\bar{s}$ are the average disadvantage caused by lethals and detrimentals, respectively.

Equilibrium population

Model I.—Incomplete dominance, h > 0:

Consider the following model (WRIGHT 1931):

Genotype	AA	Aa	aa
Frequency	$(1\text{-}q)^2$	$2q(1\text{-}q)$	q^2
Relative fitness	1	$1 - hs$	$1 - s$

We let q stand for the frequency of the mutant allele under consideration, or if there are multiple alleles, q is the sum of all their frequencies. Let u be the total rate of mutation to all mutant alleles at this locus, and s the weighted average selective disadvantage of all mutant alleles. This model is justified as a first approximation because recessive or nearly recessive mutant alleles frequently show absence of dominance in combination with each other.

At equilibrium, $q \sim u/hs$, unless h is nearly zero (WRIGHT 1931). The genetic load due to this locus in a homozygous population is then qs, or u/h (MORTON, CROW, and MULLER 1956; CROW 1958). The D:L ratio is $\Sigma(u/h)/\Sigma(U/H)$ where, as before, the capital letters refer to lethals and the lower case to detrimentals.

For $0 < h < 1$, we consider three special cases:

Ia.—h = H = *a constant:* The D:L ratio reduces to $\Sigma u/\Sigma U = n\bar{u}/N\bar{U}$, or if the average mutation rate is the same for lethals and detrimentals, n/N, where n and N are the number of loci giving rise to detrimentals and lethals. (As before, if a locus is giving rise to both, it is divided proportionately between the numerator and denominator.)[3]

Ib.—hs *constant:* The D:L *ratio is* $\Sigma us/\Sigma US$, or if mutation rate and selective value are independent, is $n\bar{s}/N\bar{S}$.

Ic.—h/s *constant:* The ratio is $\Sigma(u/s)/\Sigma(U/S)$, which, if mutation rate and selection value are independent, is $n\bar{S}/N\bar{s}$.

Model II.—Mutant gene completely recessive, h $= 0$:

The equilibrium frequency of the mutant gene or genes is $\sqrt{u/s}$. The homozygous load for this locus is qs, or $\sqrt{us}$. The D:L ratio is $\Sigma\sqrt{us}/\Sigma\sqrt{US}$. If the mutation rate is independent of the selective disadvantage, this becomes $n\sqrt{s}/N\sqrt{S}$.

Model III.—Overdominance, h < 0:

At a locus where heterozygotes are favored over all homozygotes a balanced polymorphism exists (FISHER 1930). Unless the mutation rate is of a magnitude comparable to that of selective differences between genotypes (which must rarely, if ever, be the case) the bulk of the load is not due to mutation, but to

[3] The condition that $h = H =$ a constant is unnecessarily restrictive. It is only necessary that the harmonic mean of h and H be the same and that dominance be independent of mutation rate. The condition of Models Ib and Ic may be similarly relaxed.

homozygotes that arise by segregation from the favored heterozygotes. This has been called the segregation load (CROW 1958).

We assume the fitness and frequencies to be as follows. No restriction is placed on the number of alleles, but all heterozygotes are assumed to be equal in fitness. A_i and A_j represent any two alleles, and q_i and q_j their frequencies.

Genotype	A_iA_i	A_iA_j
Frequency	q_i^2	$2q_iq_j$
Relative fitness	$1-s_i$	1

At equilibrium, $q_i = 1/s_i\Sigma(1/s_i)$ (WRIGHT 1949).[4]

In a randomly mating population the segregation load is $\Sigma s_i q_i^2 = 1/\Sigma(1/s_i) = \tilde{s}/k$, where $\tilde{s}$ is the harmonic mean of s, and k is the number of alleles at the locus. In a homozygous population, the load is $\Sigma q_i s_i = \bar{s}$. It is interesting to note that the contribution of each allele to the homozygous load is the same, i.e., $q_i s_i = \tilde{s}/k$, and that this is the same as the total load from all homozygous alleles in a randomly mating population.

IIIa.—Multiple alleles: The D:L ratio in a homozygous population is $\Sigma\tilde{s}/\Sigma\tilde{S}$, where the sums are over all overdominant loci maintaining detrimental or lethal alleles. This may be written $n\tilde{s}/N\tilde{S}$, where s is the arithmetic mean of a series of harmonic means. Notice that this is practically the same as Ib.

IIIb.—One pair of alleles, s_2 constant and much less than s_1:

With this model the load due to the more drastic allele is $\tilde{s}/k$, where k is two, and may be written $s_1s_2/(s_1 + s_2)$, which if $s_1 >> s_2$ is approximately s_2. Assuming s_2 is constant and the same for loci where s_1 is lethal as for those where it is detrimental, the D:L ratio is simply n/N. Note that this is the same as Ia.

All these models and their consequences are summarized in Table 3.

If detrimentals and lethals have the same dominance (Ia), the D:L ratio at equilibrium is larger than that in new mutants by a factor $\bar{S}/\bar{s}$. For example, if $\bar{S}$ is 1.0 and $\bar{s}$ is 0.1, the D:L ratio would be ten times as large in an equilibrium population as initially. On the other hand, if minor genes were more dominant than lethals in such a way that hs is constant (Ib), the initial D:L ratio is the same as the equilibrium value. Conversely, if minor mutants are less dominant (Ic) the equilibrium load is greatly increased over the initial.

It should be emphasized that these particular models (Ia, Ib, and Ic) are completely arbitrary and were chosen as algebraically convenient expressions for three contrasting situations. Model Ib is unrealistic for small values of s, for it admits of values of h greater than unity. However, the available data hardly justify more refined models, although others having the property of increasing dominance with decreasing s are readily devised.

Likewise many other models for overdominance could be introduced, but the

[4] This is easily seen by noting that one way of specifying the equilibrium condition is that, since the gene frequencies must remain constant, the fraction eliminated by selection has to be the same for all alleles. Thus, $s_iq_i^2/q_i = s_iq_i$ is constant. Letting $s_iq_i = C$, $q_i = C/s_i$. Since $\Sigma q_i = 1$, $C = 1/\Sigma(1/s_i)$, and $q_i = 1/s_i\Sigma(1/s_i)$.

two, IIIa and IIIb, were chosen to illustrate the fact that overdominant models exist such that the $D:L$ ratios are the same as for other models where $0 < h < 1$. Therefore, the $D:L$ ratio *by itself* cannot distinguish between partial dominance and overdominance.

COMPARISON OF THE PREDICTIONS FROM VARIOUS MODELS WITH OBSERVATIONS

Table 4 shows the total load (T), the detrimental load (D), the lethal load (L), and the $D:L$ ratio for each of the populations analyzed in this study. Similar data have been collected by other workers for natural populations as well as for new mutations induced by radiation.

The calculations given in Table 5 are based on published data of various authors who studied natural populations of several Drosophila species. Especially voluminous and useful for this analysis are the data of DOBZHANSKY and his associates.

TABLE 4

Genetic load, expressed in lethal equivalents, for the second chromosome in equilibrium populations of D. melanogaster

Population	n	Detrimental	Lethal	Total	$D:L$
Madison	231	.235 ± .029	.300 ± .051	.536 ± .049	.783
Wallace (a)	121	.185 ± .034	.272 ± .066	.457 ± .061	.680
Wallace (b)	113	.136 ± .029	.271 ± .063	.406 ± .060	.502
From weighted means	465	.180 ± .017	.287 ± .034	.467 ± .032	.627

TABLE 5

Genetic load, expressed in lethal equivalents, for several natural populations of Drosophila

Species	Chromosome	Number tested	Load: Detrimental (D)	Load: Lethal (L)	Load: Total (T)	$D:L$	Author
D. melanogaster	II	243	.345	.341	.686	1.012	GOLDSCHMIDT 1951
D. prosaltans	II	304	.179	.305	.484	.587	DOBZHANSKY and SPASSKY 1954
	III	284	.066	.061	.127	1.082	
D. willistoni	II	2004	.303	.380	.683	.797	PAVAN *et al.* 1951
	III	1166	.246	.271	.517	.908	
D. persimilis	II	106	.260	.186	.446	1.398	DOBZHANSKY and SPASSKY 1953
	III	172	.303	.194	.496	1.562	
	IV	140	.448	.175	.623	2.560	
D. pseudoobscura	II	326	.123	.157	.280	.783	DOBZHANSKY, HOLZ, and SPASSKY 1942
	IV	352	.268	.174	.442	1.540	
D. pseudoobscura	II	109	.516	.214	.730	2.411	DOBZHANSKY and SPASSKY 1953
	III	116	.421	.193	.614	2.181	
	IV	108	.346	.108	.454	3.204	
Weighted mean		...	.271	.280	.551	1.072	
Unweighted mean		...	.294	.212	.506	1.540	

The data were not always presented in a way that is adapted to our form of calculation, so that it has been necessary to make several assumptions and approximations. Firstly, 0–10 percent normal viability was made the criterion for lethality to conform with our procedure. In addition, our data are in the form of the ratio of two genotypes. However, some of the papers give the data as the proportion of the relevant class among the total, which overestimates the detrimental viabilities. If we let R stand for the ratio a/b, and P for the proportion $a/(a+2b)$, then $R=2P/(1-P)$. All the data originally given in proportions were corrected by this formula.

The $D:L$ ratio in our data is .627, whereas the weighted average for the other studies is 1.072. The data are variable, but in general the other species appear to have a $D:L$ ratio higher than we found.[5] However, the difference is not large.

These results imply that either (1) mutants with small effects occur with no greater frequency than lethals, or (2) they have more dominance than lethals and hence are eliminated relatively more rapidly as heterozygotes. It should be possible to distinguish between these two alternatives by examination of data on the $D:L$ ratio in newly occurring mutations. However, the data from various authors are not in agreement, as shown in Table 6.

In the TIMOFEEFF-RESSOVSKY (1935) data the $D:L$ ratio is .711 or 1.053, depending on the environmental conditions. An independent study on a smaller scale was done by FALK (1955), in which fitness was measured in terms of hatchability. For 56 chromosomes tested there were 3.5 times as many detrimentals

TABLE 6

Detrimental and lethal frequencies and the respective loads for newly induced mutations in D. melanogaster

Author	Chromosome	Number tested	Detrimentals		Load		$D:L$
			Lethals	Detrimental	Lethal	Total	
TIMOFEEFF-	I	432 (uncrowded)	1.9	.106	.149	.254	.711
RESSOVSKY (1935)	I	436 (crowded)	2.3	.140	.133	.273	1.053
KERKIS (1938)	I	134 (uncrowded)	2.7	...	...	...	...
		143 (crowded)	3.3	...	...	...	...
FALK (1955)	III	approx. 56	3.5	.092	.182	.274	.505
KÄFER (1952)	II	500 D_1	..	.036	.161	.197	.224
		500 D_2	..	.105	.398	.504	.264
		500 D_3	..	.166	.463	.629	.359
	I	500 D_1	..	.032	.063	.095	.508
		500 D_2	..	.013	.126	.138	.103
		500 D_3	..	.053	.195	.248	.272
	Mean	3000	.75	.068	.234	.302	.288
BONNIER and JONSSON (1955)	II	173	.82	.037	.172	.209	.215

[5] The data of GOLDSCHMIDT on *D. melanogaster*, which were gathered for another purpose, are not well suited for this type of calculation since there were no control experiments; so the value 1.012 should not be given undue weight.

as lethals, which is not significantly different from the 2–3 times obtained by TIMOFEEFF-RESSOVSKY and also by KERKIS. This is a minimum estimate, since mutants with very small effects would not have been detected. A conflicting result is represented in the work of KÄFER (1952), who examined 500 second and X chromosomes for each of three X-ray doses. The $D:L$ ratio averaged .288. Statistically detectable detrimentals were 0.75 as frequent as lethals. The results of BONNIER and JONSSON (1957) indicate slightly less detrimental effect giving a $D:L$ ratio of .215.

Assume that h is positive, ignoring for the moment the possibility of overdominance. If h is constant (Model Ia) the $D:L$ equilibrium *load* ratio is n/N, which is the same as the initial ratio of detrimental to lethal mutation *frequencies*. According to TIMOFEEFF-RESSOVSKY, KERKIS, and FALK, this is at least 2–3, perhaps considerably higher if there are a large number of mutants with minute effects. This value is much larger than the average of the $D:L$ ratios observed for natural populations.

On the other hand, if the value of h increases as s decreases the initial and equilibrium load ratios will be more similar. In fact, with the particular model (Ib) where hs is constant, they are the same. The observed $D:L$ ratios in our population (.627), which were obtained from uncrowded cultures, agree with the initial value from TIMOFEEFF-RESSOVSKY's uncrowded cultures (.711). This suggests that detrimentals are more dominant than lethals, and are eliminated *relatively* more rapidly.

This result is also in agreement with FALK's (1955) data, but his numbers are too small to be more than suggestive. ALLEN P. JAMES (unpublished) has also found evidence, in comparison of haploid and diploid yeasts, that mildly deleterious mutants are more dominant than those with drastic effects.

Nevertheless, in the light of KÄFER's results we must refrain from drawing any definite conclusions at this time. If it develops on subsequent investigation that the array of newly occurring mutations as reported by KÄFER is the more typical one, a different interpretation of the present results would ensue. Her observation of 75 percent as many detrimentals as lethals agrees well with our observed equilibrium $D:L$ ratios, giving support for Model Ia. To whatever extent her method failed to detect mutants of small effect there would be a departure in the direction of Model Ib.

At the moment there does not seem to be any way to distinguish between these two models until the discrepancy between the data on newly arising mutants can be clarified. However, there is no support from this analysis for model Ic (h/s constant). Our results imply that mutants with small homozygous effect have at least as much dominance as those of more drastic effect, and if the data of KERKIS and TIMOFEEFF-RESSOVSKY are relevant, considerably more dominance.

Possibly some of the difference in different experiments lies in the density of crowding in the cultures. Both TIMOFEEFF-RESSOVSKY and KERKIS showed that the ratio of detrimentals to lethals increased when the cultures were crowded. Possibly the nutrition was greater in quality or amount in KÄFER's experiments.

This may be an explanation of the slight discrepancy between our results and those of some others who studied wild populations. Our cultures were uncrowded, as evidenced by absence of negative correlations between different classes in a culture, and this may not have been true of other studies.

All the foregoing discussion has assumed that h is positive. It is necessary to consider the possibility that a large number of loci are overdominant. As stated earlier, the D:L ratio alone cannot distinguish between these two possibilities. However, other considerations make it seem unlikely that any appreciable fraction of loci are overdominant, if our data are correct.

MULLER and CAMPBELL (unpublished) and STERN, CARSON, KINST, NOVITSKI, and UPHOFF (1952) have published data on the amount of dominance of "recessive" lethals. In each case the heterozygotes had a viability reduction of about four or five percent, relative to nonlethal-containing controls. Since this is an average value, the possibility that some lethals are heterotic is not ruled out. HIRAIZUMI and CROW (1960) have carried out a similar study, but on lethals extracted from natural populations. These results also show a decreased viability of heterozygotes, the amount being about 2.5 percent. Furthermore, there were very few instances in which the same lethal was found more than once and none where it was present more than twice. A lethal that is heterotic, or even neutral in the heterozygous condition would accumulate in a much higher frequency than one that is disadvantageous in the heterozygote, so that this test of a natural population is much more stringent than a study of new mutants. Similar conclusions were drawn by CORDEIRO (1952) and DA CUNHA, DE TOLEDO, PAVAN, DE SOUZA, PIRES DE CAMARGO, and DE MELLO (1958). Therefore, we can conclude that the great majority of lethal genes in a population are "classical" and not heterotic, and in fact, have an appreciable heterozygous disadvantage, enough that this is the major factor in determining their frequency in the population.

If lethals are representative of mutants in general, then there are very few heterotic loci. However, the possibility remains that mutants with small homozygous effects are more frequently overdominant. Yet our data offer no support for this interpretation since the D:L ratio is low. If lethal mutations were partially dominant, but there were an appreciable number of overdominant loci with small homozygous effects, they would accumulate in the population in large numbers and inflate the D:L value. Since this value is already small, there is not room for very such many loci. For as their number increases, we must postulate still greater dominance or still smaller initial numbers of mildly deleterious mutants with $h > 0$. The most probable conclusion from our data is that heterotic loci make a relatively small contribution to the homozygous genetic load.

The suggestion that a major fraction of all loci may be heterotic has been made by WALLACE (1958) on the basis of a study of radiation induced mutations in which he finds that flies with a pair of chromosomes that are identical except for the fact that one has been radiated have a higher viability than flies with the same chromosomes without radiation. Presumably such radiation-induced mutants would be deleterious to some extent if made homozygous. Why do these not show up as a contribution to the homozygous load in our analysis?

The answer is not clear. As stated earlier, there is no evidence in these data that overdominant loci are making any appreciable contribution to the homozygous load. Since each locus of this type would make a disproportionately large individual contribution to D, this argues that the fraction of such loci must be very small. A population in which there is an overdominant locus where the harmonic mean homozygote viability reduction ($\bar{s}$) is .01 will have a homozygous load of one percent irrespective of the number of alleles. This follows from the formulae given under Model III. It would require 50 "classical" loci with $u = 10^{-5}$ and $h = .05$ to produce the same homozygous load, since this is u/h for each locus. This means that a very small minority of heterotic loci could cause a substantial reduction in homozygous viability. Furthermore, unless the number of alleles is very large, they would also cause a large reduction in viability of a randomly mating population. Of course, there may be many overdominant loci with extremely minute s values which would be impossible to detect by these methods. The question then becomes the number of nearly neutral alleles that can be maintained against random fixation.

The argument that only a small fraction of loci are overdominant does not imply that these are not important in a randomly mating population. On the contrary, a small minority could still be a major factor in determining the mean and variance of the population fitness.

Our analytical procedure is based on the assumption that different mutants act independently. There may well be appreciable interaction, particularly synergism such that multiple mutants are eliminated at a faster rate than if each one caused an independent probability of death. TH. DOBZHANSKY, LEVENE, B. SPASSKY, and N. SPASSKY (1959 and several earlier papers) have shown evidence for homozygous epistatic effects revealed by recombination between chromosomes from natural populations, but the data do not permit any estimate of the magnitude and direction of such interactions. More relevant to our problem are existing data on the linearity of inbreeding decline in yield of corn (NEAL 1935), various characters in guinea pigs (WRIGHT 1922), and litter size and weight in swine (DICKERSON, BLUNN, CHAPMAN, KOTTMAN, KRIDER, WARWICK, and WHATLEY 1954), which argue against such synergistic effects being large. On the other hand, STRINGFIELD (1950) reported that with certain maize hybrids backcrosses showed higher yields than F_2 populations, indicating some complementary interaction. The point could be tested further by comparison of homozygous and heterozygous chromosomes with different levels of background heterozygosity in the other chromosomes, or tests of combinations of mutants. Pending more explicit evidence that synergistic effects are large, the assumption of independence seems to us to be a reasonable first approximation.

SUMMARY

Four hundred and sixty-five second chromosomes were extracted from a natural population of *Drosophila melanogaster* and from a large population cage that had been maintained for several years. A comparison of the viability of homozygous

chromosomes and random nonidentical pairs revealed that the homozygotes had a viability reduction or genetic load of 47 percent. The ratio of the load due to mildly detrimental mutants (D) to that for lethals and near lethals (L) was .627. These results imply that either (1) mutants with small effects occur with no greater frequency than lethals, or (2) they have more dominance than lethals and hence are eliminated relatively more rapidly as heterozygotes.

Comparison of the D:L ratio in these populations, assumed to be somewhere near equilibrium, with that for newly induced mutations can give information on the comparative rate of elimination of lethals and detrimentals. The data are insufficient to distinguish between greater dominance for genes of small effect and the same average dominance for lethals and detrimentals. However, the data offer no support for the idea that mildly detrimental genes are less dominant, or for any substantial contribution to the homozygous load from overdominant loci.

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